Disperse Blue 1

CIR EXPERT PANEL MEETING
DECEMBER 13-14, 2010
Memorandum

To: CIR Expert Panel Members and Liaisons

From: Monice M. Fiume MMF
Senior Scientific Analyst/Writer

Date: November 18, 2010

Subject: Disperse Blue 1 Draft Amended Report

In 1995, the CIR Expert Panel published the Final Report on the Safety Assessment of Disperse Blue 1, which concluded that this ingredient is safe for use at 1% in hair dyes. At the April 2010 Expert Panel meeting, the Panel opted to re-review Disperse Blue 1. At the August meeting, the Panel reviewed the Draft report, and the report was tabled for the determination of margin of safety (MOS) for the use of disperse blue 1 in hair dyes. A quantitative cancer risk assessment has been provided to the CIR, and is included for your review.

The carcinogenicity and risk assessment text from the 1995 report on disperse blue 1 have been included in this re-review so that the relevant data are readily available. The risk assessments have been performed using varying assumptions, but all seem to point to a notable margin of safety for the use of disperse blue 1 in hair dye. While the mode of action for induction of smooth muscle tumors is not known, several risk assessments assume a genotoxic or other non-threshold mechanism of carcinogenesis to provide the most conservative estimate for a margin of safety. The results suggest that the margin of safety is sufficient to allow use in hair dyes.

Dr. Cohen’s presentation from the August meeting is also included as supporting documentation.

The following options are available to the Panel:
1. the Panel can reaffirm its original conclusion on disperse blue 1 and decide not to re-review disperse blue 1; or
2. the Panel can continue with the re-review of disperse blue 1, and, based on the new risk assessment data, determine a new maximum use concentration; or
3. the Panel can state that it is still not comfortable with the carcinogenicity data and the uncertainty regarding the mode of action of the smooth muscle tumors, and determine that there are insufficient data for a determination of safety; or
4. the Panel can state the it still not comfortable with the carcinogenicity data, and determine that disperse blue 1 is unsafe for use in cosmetics.
SAFETY ASSESSMENT FLOW CHART

Public Comment

CIR

Expert Panel

Re-Reviews

Report Color

**The CIR Staff notifies the public of the decision not to re-open the report and prepares a draft statement for review by the Panel. After Panel review, the statement is issued to the Public.**

**If Draft Amended Report (DAR) is available, the Panel may choose to review; if not, CIR staff prepares DAR for Panel Review.**

- Draft Priority List
- Draft Priority List
- ANNOUNCE
- Priority List
- INGREDIENT
- SLR
- Decision not to reopen the report*
- Draft Report
- ISD Notice
- Draft TR ISD
- Tentative Report
- Draft FR
- Final Report

DRAFT PRIORITY LIST

Is new data cause to reopen?

DOES NEW DATA SUPPORT ADDING NEW INGREDIENTS?

DRAFT REPORT

DRAFT TENTATIVE REPORT

DRAFT FINAL REPORT

Issue FR

Table

Draft Amended Report

Tentative Amended Report

Draft Amended Final Report
Question of Re-Review: April 4-5, 2010

In 1995, the original safety assessment on Disperse Blue 1 was published, with a conclusion that this ingredient is safe at up to 1% in hair dyes. The question of whether or not to initiate a rereview was presented to the Panel. The Panel was informed the use of Disperse Blue 1 had dropped from 112 uses, in 1994, to nearly 0 in 2009. (There were 3 uses according to FDA VCRP data, but according to an industry survey, there was no use.)

While very little new published data were available, NTP’s 11th Report on Carcinogens and IARC’s overall evaluation for Disperse Blue 1 were included in the re-review document. Both stated that Disperse Blue 1 can be possibly carcinogenic to humans.

Even though it appears that Disperse Blue 1 is not in use, the existing CIR conclusion does state that this ingredient is safe at up to 1% in hair dyes. Therefore, the Panel decided to open the re-review to re-evaluate the carcinogenicity and risk assessment data.


Additional clarification as to why Disperse Blue 1 is not permitted for use in cosmetics in Europe has been added to the report. A search performed on June 1 did not find any additional relevant published data.

At this meeting, the Panel tabled this report. The unusual tumors that resulted in a carcinogenicity assay were discussed, following an industry presentation by Dr. Sam Cohen. The report was tabled, as the Panel requested margin of safety data.


An updated Risk Assessment was provided to the Panel. CIR provided further analysis.
CIR HISTORY OF DISPERSE BLUE 1 (88a-84)

Scientific Literature Review: announced March 22, 1993

Technical Analysis: August 16-17, 1993
No unpublished data were submitted during the 90-day comment period following the release of the Scientific Literature Review on this ingredient.

CIR did receive comments regarding the wording used in the Epidemiology section of this report. This section is the standard summary used in all hair dye reports. After a careful review, it is clear that this standard must be updated to include more recent studies. CIR is in the process of revising this section and will include it in this report, as well as other hair dye reports, when the new update is completed.

Both Teams agreed that no additional data were needed to evaluate this ingredient. Although there was evidence that Disperse Blue 1 is an oral carcinogen, negative results were obtained in a skin carcinogenicity study. They noted that this ingredient is a sensitizer.

Draft Report: November 22-23, 1993
The Panel was concerned about the potential carcinogenicity of Disperse Blue 1. The Panel did not want to rely on the dermal carcinogenicity study (Jacobs et al., 1984) because it lacked details. Since the Panel was informed that Disperse Blue 1 is used only in non-oxidative hair dyes, it was agreed that cutaneous absorption of this oral carcinogen is necessary.

The Panel tabled this report and informally requested:
(1) cutaneous absorption data
(2) any assessment of carcinogenic potential

A bio- and mathematical-risk assessment of hair dye use is to be supplied by Dr. Corbett of Clairol, which may address these issues. However, if these data are not available or adequate, the Panel would expect to request:
(3) dermal carcinogenicity study in rats using the protocol of the NTP.

Draft Report: February 28 - March 1, 1993
No data or commitments to provide data were received since the last Panel meeting.
Updated search: Nov 8, 2010
2475-45-8 on Toxline
1 found

Updated search: June 1, 2010
2475-45-8 on Toxline/Pubmed
3 found/0 useful

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Disperse Blue 1 (2475-45-8)
______________
Nov. 29. 2009


PUBMED
Medline - 7

TOXNET
Toxline - 77
ChemID Plus - 1
NIOSH -
NIST -
DART - 1
HSDB - 1
CCRIS - 1
Genetox - 0
CPDB - 1

STN (2/2/10) – nothing new to add

FDA Frequency of Use - 3
EWG (2/18/10) - 0 uses

EAFUS - 0
GRAS -
IFA - 0
OTC - 0

EU - Annex II/700

NTP - x (11th Rpt)
IARC - 1
WHO - x (IARC paper)

USP - 0
OECD - 0
HPV - 0
Merck - 0
AFTERNOON SESSION

(1:01 p.m.)

DR. BELSITO: Dr. Cohen is here. Okay. Then I think in the interests of his travel schedule we'll look at Disperse Blue 1. And the issue here as you've heard is that we previously looked at this in another life and found it to be safe as used. But at the April meeting we decided to reopen the safety assessment to readdress the carcinogenicity issue, particularly in light of the information that we had that the European Union had not approved this for use as a hair dye and as a result of the NTP carcinogenicity study.

So that's what we're doing at this point. And the question is based upon our relook at the material, what we heard from Dr. Cohen this morning, particularly in light of the smooth-muscle tumors and the inconclusive evidence as to whether this is or is not the human carcinogen, whether we feel we want to reopen and change our conclusion to insufficient data.

Now, having heard from Julie that there's apparently no reported uses, if we did that it's not likely that we're going to get any data back on it. But now that we've bifurcated the insufficient use to insufficient use with actual reported uses versus insufficient use without reported uses, I think that's a non-issue.

So having said that I'll turn it over to Paul and Curt for comments, and Dan, about the carcinogenicity.

DR. BRESLAWE: I want to point out that we have three reported uses.

DR. BELSITO: We have three reported uses.

DR. BRESLAWE: According to FDA.

DR. ANSELL: But the database has been corrected.

DR. BRESLAWE: Oh, it has been. Wonderful.

DR. ANSELL: To one reported use. We had contacted that person and they say that that is an error. They do not use it. So right now we find ourselves in that situation. There are no reported uses.

DR. BELSITO: All right.

DR. ANSELL: And indeed, we heard this morning it's not surprising since there's none commercially available.

DR. BELSITO: Paul, Curt, Dan?

DR. KLAASSEN: Well, I -- I think there is enough concern here, you know, while there's probably not much of this chemical that's going to be absorbed. It's one of the reasons that you don't feel too bad about it. But in contrast to a lot of the other type chemicals this one is different I think in two ways. One is that this one has about as many positive mutagenic tests and negative mutagenic tests where many of these compounds we've looked at in the past have had just about all negative mutagenic tests. So that's one thing that's quite different.

And the other is that this does not appear to be a simple calculi in the renal system in the bladder, et cetera, but it's also in the muscle. So I think I would go along with inadequate data, insufficient as a conclusion.

DR. BELSITO: In your data need there's more data on the mechanism of the action of the carcinogenicity?

DR. KLAASSEN: Right.

DR. BELSITO: Paul?

DR. SNYDER: Yeah. I concur with Curt. I think we're obligated to go insufficient here because we don't know the smooth-muscle tumor pathogenesis. While the epithelial pathogenesis is pretty well understood with the formation of calculi and unique to the rat and that's known across a broad range of chemicals, but the caveat here is the (unintelligible) tumor, in particular the smooth muscle, because it doesn't go with the -- Dr. Cohen alluded to it this morning -- doesn't go with the typical type of tumors we see with foreign body-type reactions with those being mostly fibrosarcomas and fibromas. This is unique and this is -- these are specifically smooth-muscle tumors. So I think we need to know and understand that a little better before we can
go any further than that.

DR. LIEBLER: So I'm in the same place as you guys. I'd like to thank Dr. Cohen for his excellent report. Both his written and oral report this morning really helped eliminate some of the data gaps for me. And I agree, I could not support a reaffirmation of the original conclusion.

DR. BELSITO: Okay. So we're going to reopen this and we're going to change our conclusion from safe as used to insufficient data. And the data we need is more carcinogenicity data, particularly looking at the method of induction of these smooth-muscle tumors. Correct?

DR. LIEBLER: Yes.

DR. BELSITO: Okay.

MS. FIUME: Does it need to be clarified whether it's oral or dermal or what method is done in the study? Does that matter for the data need?

DR. BELSITO: Well, we know absorption at least. So, I mean, I think oral or dermal. I mean, dermal would always be best, you know, because the way the product is used. But we could do oral and do the math.

MS. FIUME: So I can leave it unspecified?

DR. BELSITO: Right. Yeah.

MS. FIUME: Okay.

DR. BELSITO: You can leave it unspecified because the reality is I think we'll all be surprised if we have that data need since it doesn't seem to be used anymore.

Okay. Dr. Cohen, any comments?

DR. COHEN: No. I would just add the insufficient information about the genotoxicity and the (inaudible) carcinogenicity.

DR. BELSITO: The insufficient information about the genotoxicity and then I missed the rest.

DR. COHEN: The nitroanthraquinone contaminant.

DR. BELSITO: Okay. In the discussion.

DR. COHEN: (inaudible)

Most of those other anthraquinones also cause liver damage, right?

DR. COHEN: Yes, yeah.

DR. BELSITO: Julie, any comments?

MS. SKARE: No.

DR. BELSITO: Okay. Good. So now we're turning to our original schedule, dicarboxylic acids, which is Pink 3, also known as sebacic acid. We tabled that last December to reorganize the reports into acids and esters. And we agreed to delete oxalic acid and so the report has been rewritten. And there's a feature on diester metabolism. And we can ask Dan to comment on that. Although we tabled it, we sort of did this hint hint to industry at that time that there were additional data that we'd want to see, including impurities data for a representative long and short chain dicarboxylic acid. And for a short and long chain diester. And dermal penetration, there could be octanol-water partition coefficients and information on irritation and sensitization and use concentration.

MS. FIUME: Dr. Shank, I believe it's Table 1 now has the definition and it's the dictionary monograph, so I will make sure that it is referenced there. But it is... it's now been added to the cosmetic ingredient dictionary and handbook.

DR. SHANK: Okay, thank you. Thank you.

DR. MARKS: Okay, I will then move tomorrow that a final report on safe is issued for the stearyl heptanoate group and we'll move on now to, I believe is the last ingredient we're going to look at today, that's Disperse Blue 1. And I don't know if the presenter is still around from this morning.

DR. ANDERSEN: No, Dr. Cohen had to catch a plane.

DR. MARKS: So, in 1995, the expert panel published a safety assessment with Disperse Blue, safe as use, at 1 percent in hair dyes. In the April meeting, there was concern about this conclusion and, therefore, it was reopened. This was based on concern with the NTP, the IARC...
Classification, banned in the EU. Despite that, our team still felt that it need not be reopened and I think that issue still stands now as do we want to continue to reopen and proceed with a new conclusion or do we want to not reopen? Is there enough new data or is there any data suggesting --

DR. SLAGA: Same conclusion, yet.

DR. MARKS: And as you recall, in April, their reason for not reopening was that it was based on the absorption data, lack of absorption and then a negative dermal tox in mice. And we were able to explain the rat studies which created the concern of carcinogenicity to humans.

So, where do we want to go with this? Do we want to not go back and reaffirm our conclusion in the last meeting not to reopen or do we want to reopen?

DR. SHANK: Do not reopen.

DR. SLAGA: Do not reopen.

DR. MARKS: Well, Ron?

DR. HILL: I agree.

DR. MARKS: And -- thanks, Wilma. John, do you have any comments? And I'll even ask Linda this because this is obviously a controversial issue, meaning with these other rulings.

MR. BAILEY: Well, I think we heard a very comprehensive presentation this morning that went over the existing data and where that stands. We did query the FDA voluntary database. We found one product that was registered and have subsequently confirmed that that product is not accurately registered and is being withdrawn or has been already withdrawn. So, it sort of falls into the zero use category from our perspective.

I think the challenge for the panel is, are you comfortable with, you know, in light of the presentation today, of continuing it as a safe as used ingredient and that's your call, of course, but nevertheless, I think there was a very thorough and, I think, very objective presentation of the data from this morning.

DR. MARKS: Alan, do you want to comment? Because I think that at least in my notes I like the phrase, "Where's the beef?"

DR. ANDERSEN: Yeah, this one is really tough. From a resource standpoint I'm not sure I can get hugely excited about wasting any more of Monice's time on this. If we went -- if we believed that there's still some doubt and went to amend this to say "insufficient data," then -- and it's for an ingredient that has no current reported uses, what did we accomplish?

DR. HILL: Moreover, it seems that the ingredient is not even manufactured anymore. If that's true then --

DR. ANDERSEN: Also an issue. Getting it right has some value, but I'm hearing an unambiguous assessment that the dermal penetration, the exposure levels in the face of the only data being very high exposures, does not produce a reasonable risk assessment that there's anything here for humans.

Having said all of that, being somewhat philosophical for a moment, this is exactly the issue that folks who espouse the precautionary principle salivate over. What is it about this carcinogen that you guys don't get? And that would be the position that's established and a failure to at least acknowledge that there's some uncertainty -- Europe has gone all the way to saying don't use it in cosmetics. You could go as far as to say insufficient data. I think it becomes a philosophical difference.

From a risk assessment standpoint, I think the panel got it right the first time. So, I don't disagree, Ron and Tom and Ron, with anything that you said in your very positive statement to not reopen. From a risk assessment standpoint, you're right.

DR. SLAGA: We can deal with the lot which you brought up in the discussion. And we should.

DR. ANDERSEN: Yes, we can, but -- and we didn't for -- when it was done, we didn't do a half bad job the first time around, but we still have an issue of at high exposure levels a clearly
A carcinogenic chemical that has produced at least one tumor type that is unusual and not routinely seen. And where have we accommodated any element of protection for the consumer by saying that it's safe to use? So, it's just that -- applying that different perspective of precautionary principle would lead us to say, no, we don't think the data are sufficient. It isn't a lock.

Applying risk assessment, what's the exposure actually going to be? And could it ever attain the level shown to be carcinogenic? Not a chance. I think you're perfectly comfortable.

DR. MARKS: Tom, I'm probably going to ask you tomorrow if the other team has a differing conclusion whether or not to reopen to expound upon why we -- just those points that Alan's made. Is there any -- with Alan talking about, again, a sort of broader picture, not just a purely scientific risk assessment picture, is there any concerns, again, with not reopening it? And actually, Rachel, I'll ask you because a lot of what Alan is talking about is the perception that comfortable with, I think is the way to answer it.

DR. MARKS: Okay, thank you, Rachel. Thank you, Alan.

Does -- so, I'm going to move tomorrow that we do not reopen, but we will have a robust discussion with the rationale why not to reopen. And, Tom, when the other team suggests that we put an insufficient data announcement, I ask for your rationale on that discussion. I don't know what the other team's going to do obviously, but I'm anticipating there may be a disagreement. And I think ultimately the decision will be made in what we feel is the safest for the public, obviously.

DR. MARKS: Okay, thank you, Rachel. Thank you, Alan.

Does -- so, I'm going to move tomorrow that we do not reopen, but we will have a robust discussion with the rationale why not to reopen. And, Tom, when the other team suggests that we put an insufficient data announcement, I ask for your rationale on that discussion. I don't know what the other team's going to do obviously, but I'm anticipating there may be a disagreement. And I think ultimately the decision will be made in what we feel is the safest for the public, obviously.
ingredient that we had previously found to be safe as used as a hair dye. And looking at the data, particularly an NTP study, we decided to reopen this report. Having done that, my team was a bit concerned. Previously we were aware of epithelial bladder carcinoma that we thought was due to formation of calculi in the bladder that would not be pertinent to humans, but in the concurrent report we were made aware of some smooth muscle bladder tumors as well, and the mechanism of those is unclear to us. And so at this point, I think that we need to proceed with an -- change our opinion to insufficient data. And the insufficiency is understanding the mechanism of the bladder carcinoma, particularly smooth muscle.

DR. BERGFELD: That's a motion.

DR. BELSITO: That's a motion.

DR. BERGFELD: That's a motion? Is there a second? Or further discussion?

DR. MARKS: There is not a second from our team. Our team heard that excellent presentation yesterday morning, the concern about smooth muscle tumors. Yet the margin of safety, we felt, was okay. This was all data from 1986. And so we felt that we could continue despite the concern of other colleagues, particularly in the EU, that this should not be reopened and that these concerns would be robustly discussed in the portion of the not reopen review. And Tom, do you want to add any more comments?

DR. SLAGA: Well, at the last review we essentially had everything we needed, including risk assessments. And if you take everything in consideration, the level that is applied or used -- matter of fact, there's no use right now and it's not even manufactured. But 50 percent of the formulation is related to Disperse Blue 1. The -- there was a second study, Burnett and Squire, that actually used more mice than the NTP study and they didn't get any of the smooth muscle tumors. And granted they did get the bladder tumors. But if you look at the dose relationship, their dosage below that that did not get it. And you calculate the risk assessment, there's really significant -- in the risk assessment there is, you know, amount that is needed to reach that where there is a safety -- extensive safety margin with that. So we felt that there wasn't anything really new and that there was no reason to reopen it. Number one, it's not even manufactured. Number two, it's not even used. And with the safety margin we felt that we could go with it.

DR. BERGFELD: Dr. Snyder?

DR. SNYDER: Yeah. I appreciate that Ron's -- and -- I'm sorry, Tom's comments. I guess the complicating factor is that we have this report out there with a 1 percent limit, and we've asked last time for justification on the 1 percent limit. I don't believe that we received the basis for the 1 percent limit. In both the 1993 report and this report, the reported uses that we received to date are .65 percent. And so we're putting a limit on an ingredient that we probably generally agree is a very weak carcinogen at higher than the use -- current use. And so that's -- it's more of a -- in a re-review process, are we still comfortable with having a limit that's higher than the current use?

The other issues pertain to the -- as Dr. Cohen presented yesterday -- the incidence of smooth muscle tumors in rodents is very, very, very rare. And the fact that these spiked up in these animals in association with the calculi and the presence of the compound are of concern. We have a NTP report that keeps -- keep getting put out annually that list it as a reasonably anticipated to be a carcinogen in people. We have an IARC report that says that it's a plausible carcinogen in a group B designation. And so I think if we're all comfortable with the fact that in it's current use under current conditions that the risk is negligible, then I think we would be okay. But I don't think that we've adequately addressed the limitation -- the 1 percent limitation.

DR. SLAGA: Well, the limitation was in the minutes and it was based on absorption.
Actually, our group at the time wanted a limit of .3 based on the dermal carcinogenicity --

DR. SNYDER: Right.

DR. SLAGA: -- because it was totally negative. And -- but if you take into consideration that it's a very poorly absorbed -- applying the 50 percent of it and only a couple percent get absorbed, you know, you have quite a safety margin on top of it. The lifetime exposure risk assessment was very, very liberal when applying it for the life of a human many times per year. And if you take that all in consideration, the 1 percent still is -- there's quite a margin of safety for this compound. And the fact that it doesn't cause any dermal cancer eliminates -- there was a few bit of data suggesting that it was mutagenic in some cases. Not in a similar study looking at the same assays, but if you look at the total skin studies. It really says it's not carcinogenic, it's not absorbed very much, so the amount that could get in possibly to even potentially cause kidney cancer is extremely, extremely small amount.

DR. SNYDER: Yeah, I agree. The dermal studies were at .1 percent and .3 percent, so they -- that was a significant difference.

DR. SLAGA: Yeah.

DR. SNYDER: And even in the discussion of the Burnett, they talk about it potentially being a threshold effect in regards to the induction of the bladder tumors.

So, I guess I'll refer to my other colleagues for their comments.

DR. BERGFELD: Dr. Klaassen, can you give us a comment?

MR. KLAASSEN: Well, this is, you know, kind of a borderline one as far as I'm concerned. The fact that we are -- one thing that was different between this chemical and some of the other dyes that we've looked at is the mutagenicity is much more -- I mean, there were many positives and many negatives. And so that gives one a little bit more concern than some of the other dyes where almost all of them were negative. And I'll agree that the percent that's absorbed and the likelihood of ending up with cancer is very low. But the fact that, you know, the mode of action, first of all, is that you're getting it in these muscle cells which is quite different. And the second is that it seems to be more mutagenic than some of the others. Not a strong mutagen, but it was enough to kind of throw me on one side of the fence rather than the other side.

DR. SLAGA: Well, if the normal sequence if it is mutagenic, then we have to have it tested as a skin carcinogen, which we have. And it comes out negative in a very long skin tumor genesis study. So, I think one has to take that in consideration the --

DR. BELSITO: Well, I mean -- but as you're already mentioned, that study was at .3. And the existing regulation is at 1 percent. So if you use that argument, then we need to reopen it to reduce the -- the limit on concentration.

DR. SLAGA: We made that suggestion in 1994, I believe, so.

DR. BELSITO: You were prescient --

DR. SLAGA: We were pressured by you to --

DR. BELSITO: You were prescient, not pressured.

DR. BERGFELD: Dr. Shank, do you care to make a comment, please?

DR. SHANK: What kind of data would you need? You said insufficient data, and you need mode of action. Just what kind of experiments do you have in mind that would provide the data that you want?

DR. SNYDER: I guess I'm less concerned about the -- about whether we go insufficient or we don't reopen. I'm more concerned about the 1 percent limit. And I guess if everybody's comfortable with that 1 percent limit related to the risk assessment and the absorption and that's going to be very clearly detailed in the discussion, then that would -- I would -- I could go that way.
I mean, if not -- if we're going to lower it, then I think we have to have some more justification as to why we're going to lower it and maybe some -- if we're going to keep it that high, then maybe we have to have justification as to what potential mechanism of action is going to be. And that's going to be very problematic because I don't know how you would exactly design that study, other than repeat the study. The Burnett study, the repeat study, was not as long a duration. And so as Dr. Cohen stressed yesterday that these are late in life occurring tumors, and so there -- again, related also to the threshold effect, that that may potentially be a confounding difference between the two. So.

DR. MARKS: To me, the options are either not reopen or unsafe. And if we can't get to unsafe, then for an ingredient which has no uses at this point, it's hard for me to expend the effort to reopen and revisit this. Particularly when if we declare it unsafe we have to come up with a scientific reason. If we don't have a margin of safety to support that, then I think we have problems.

DR. BERGFELD: Dr. Bailey?

DR. BAILEY: Yeah. I have a concern that -- I mean, right now we have an opinion that it's safe at 1 percent and the -- so the question is, should that stand as is or should it be reconsidered. And if it's reconsidered, then you have the usual outcomes; it's safe, it's not safe, or it's safe with conditions. It really makes me nervous to talk about ingredients that have no use or are not being made because that's not really -- I mean, I understand the resource implications, but the net effect is going to be if it's not reopen, then the public will see a conclusion of safe at 1 percent and someone, you know, may pick this up and decide to manufacture a product. There's nothing that would prohibit, restrict that step.

So I think that Paul, your point is that if the data supports a different use level or a different outcome, then we should consider that.

And I don't think it would be too labor intensive, because we have a very concise data set that could be brought to bear and brought -- and pulled into the report. So I think it's really important not to make the decision because it's not used or it's not being manufactured. I think you really need to look at the conclusion that's there and whether that's an appropriate conclusion based on the science and what we heard yesterday.

DR. MARKS: Can I distill what you just said? So, you're recommending it stay as a reopen document and to reduce the limitation to 0.3. Is that what I heard you say?

DR. BAILEY: No, that's not what I said.

DR. MARKS: Okay.

DR. BAILEY: I said that's among the options. I mean, you know, we did hear some concerns about the soft tissue effects and what that means under use conditions. Perhaps you would even want to calculate a margin of safety if that's something you could do with the data you have. But I just think, you know, if we don't reopen and change then the existing conclusion is what the public will see. That's the official position of the CIR expert panel. And you just need to be very comfortable with that, I think.

DR. BERGFELD: Dr. Slaga, you want to respond? You want to repeat the margin of safety information?

DR. SLAGA: If we reopen it even just to change the down to 0.3, what do we gain? We gain a factor of 3. -- something in the risk assessment, greater safety. If that's -- we can justify that. I don't -- you know, the margin of safety is much better than most compounds we deal with. So I don't know where that would take us.

DR. BERGFELD: Dr. Belsito?

DR. BELSITO: Well, I mean, I would agree with what John said. If we don't reopen it then we're saying it's safe as used up to 1 percent. And not to beat a dead horse but, you know, we have one study where there are no mesenchymal tumors, we have another study where there is. Off the top of my head, I don't know
what the NOAEL is in the study where you saw the mesenchymal tumors versus the study where you saw the bladder tumors based upon calculi.

But, you know -- and if I'm not a, you know, carcinogenesis expert. But if when you see effects like cancer endpoints and the usual next step is to do a dermal carcinogenicity study, well, that's been done at .3 percent. We don't have a dermal carcinogenicity study at 1 percent. So I would be very uncomfortable keeping that as is because again, as Dr. Bailey said, someone can pick up our report and say, okay, safe to use Disperse Blue in the U.S. at up to 1 percent. And I'm not certain that that's the case. So if you don't feel that we can go insufficient for further information about these smooth muscle tumors, I would be much more comfortable going with a new conclusion that it's safe at .3 percent, recalculate the margin of safety based upon the new data from NTP, and see what that comes out as.

DR. SLAGA: I would accept that. Can we just ask -- say we made a mistake and go back in and change it to?

DR. BELSITO: That's what re-reviews do all the time --

DR. SLAGA: Because I was concerned last time.

DR. BELSITO: We did that with one chemical -- I'm blanking on the name -- where we picked up that the data supported it only up to .1 and not 1 percent.

DR. SLAGA: I would go with .3. That does -- if we're allowed to do that.

DR. BERGFELD: Dr. Marks and Dr. Shank?

DR. MARKS: I will then, for our team, second the motion to reopen. Or, I mean -- excuse me. Your motion, then, Don, was to go with an insufficient data.

DR. BELSITO: Our motion was to go with an insufficient data. What I would recommend that we do at this point is table it, assume that we're going to go with a safe as used at .3 percent, redo the margin of safety, let's make sure that that looks good to everyone and everyone is comfortable with the new document. But where we're going with this is probably a safe up to .3 percent in hair dye products.

DR. BERGFELD: So you're actually going to amend this?

DR. BELSITO: We're going to amend the conclusion.

DR. BERGFELD: Thank you.

DR. SHANK: And how do you handle the issue of smooth-muscle tumors?

DR. BELSITO: Do what?

DR. SHANK: Smooth-muscle tumors? How do you -- that's your concern as the carcinogen --

DR. BELSITO: Right.

DR. SHANK: How do you handle that?

DR. BELSITO: We're going to use a risk assessment, looking at the NOAEL for that as well as the other NOAELs that we had used before.

DR. SLAGA: The NTP study, the second dose level didn't have any if I remember correctly. So it should be calculated.

DR. BERGFELD: So you -- there's been no second to your first motion. There -- now has a second motion to table and there's no -- oh, is there a second to table?

DR. MARKS: Second.

DR. BERGFELD: All right. There's not discussion on the table, so all those in favor of tabling with the intent of making an amended conclusion please indicate by raising your hands.

Unanimous.

Thank you. Very robust discussion. Thank you very much.

The next ingredient is Dr. Marks, the dicarboxylic acids.

DR. MARKS: Re, in December of 2009, this group of cosmetic ingredients under the dicarboxylic acids were tabled so that the report could be reorganized. And now we have the acids, the salts, and the esters. They've been separated in this report. It's a large number -- if you look on the introduction on CIR panel book page 48, you see the number of ingredients. And with that in mind we move to issue a tentative final
ingredient and that is the Disperse Blue with Dr. Marks.

DR. MARKS: In 1995, the CIR Expert Panel published a safety assessment of Disperse Blue 1 with the conclusion that it’s safe as used in hair dyes at a concentration up to 1 percent. In that report there was the issue of possible carcinogenicity to humans and that was so noted and discussed in our team. However, we thought that there was no new data to suggest a change in the conclusion so we moved not to reopen this report.

DR. BERGFELD: With that motion is there any discussion or a second? Dr. Belsito?

DR. BELSITO: We actually thought that we should reopen it to more thoroughly review the data particularly in light of the fact that HC Blue or Disperse Blue No. 1 has been banned in Europe and to look at further the data that they looked at. We acknowledge the fact that it doesn’t appear to be used currently in the U.S., but if we don’t reopen then we’re stating that it could safely be used up to 1 percent. And perhaps that in fact is the case, but we felt we should reopen to do a thorough review of the data in light of the fact that if we do say it’s safe up to 1 percent that will be somewhat controversial because we will have differing opinions on either side of the Atlantic Ocean as to the safety of this ingredient.

DR. BERGFELD: Dr. Slaga?

DR. SLAGA: Since there was no new data, all the change related to its possible carcinogenicity is by one review group related to the European standard so to speak. There is nothing in here that would suggest that there is any change over what we recommended before and we set limits based on --

DR. BELSITO: I guess that’s true. We did get data that apparently we didn’t look at before even though it was an FDA analysis of the absorption of this chemical and then a safety assessment that depending upon on how you think this acts, whether it is genotoxic or whether it has a nongenotoxic mechanism of carcinogenicity due to the formation of renal calculi depending upon what you believe that mechanism to be changes significantly the safety assessment and I’m not sure that we really adequately addressed that. Again, this is not my area of expertise. I think our group was more swayed by the fact that there has been a change in IARC’s view of the potential carcinogenicity of this and we should go out and re-review it and if in fact after that we feel that we don’t need to reopen it, at least begin the process to reopen it and relook at the data.

DR. BERGFELD: Dr. Shank and then Dr. Katz if you don’t mind.

DR. SHANK: By the oral route to rodents it is a carcinogen. It was tested dermally for dermal carcinogenicity and it was totally negative. So I don’t see how reopening it and considering the same data again is going to make any change.

DR. BERGFELD: Dr. Katz?

DR. KATZ: I’m not sure I’m going to be able to help to shed any additional light. In the group where we sat yesterday, the discussion really is if you do leave it, then you are leaving it open for its safe use in a cosmetic and the question really truly remains do you really believe that it is safe. If you truly believe that it’s safe then I’d suggest leaving it alone. If the data is such that it’s not compelling that it is safe then I might suggest opening it up and going back to look again at the state of what’s out there including going back to see what the Europeans used to make their decision that it would be on their banned list. Whether or not it’s on the European list because when they called for their data that industry chose not to give the data or whether or not the data that they received show that there is something there that would make it unsafe is unknown. That’s really the question and I don’t know the answer to that portion of the question either.

DR. BERGFELD: Dr. Bailey?
1. DR. BAILEY: I think those are all very good points and I think that the idea of reopening it just to sort of validate since there is a carcinogenicity question I think is a reasonable step to take by the panel.

2. DR. BERGFELD: Dr. Snyder?

3. DR. SNYDER: I think it's part of due diligence. Just because we'll reopen doesn't mean that we're going to do anything, we're just going to reopen and take a look, reaffirm that we're comfortable with all the data that we have, there is some new absorption data and go down that pathway just doing good due diligence because there may be potential for this to be a discrepancy between the E.U. and the U.S.

4. DR. BERGFELD: Dr. Marks?

5. DR. MARKS: Of course we're always going to side with safety, so I'll withdraw my motion to not reopen it and let's reopen it, look for more data and then depending on the results of that we'll maybe not reopen again, but for the time being, reopen.

6. DR. SNYDER: Just to add, I fully agree with Ron. I think that we understand the mechanism in the rodents and it's not an issue so I don't want that to be overly highlighted.

7. DR. BERGFELD: Ron, do you want to make a comment? Dr. Shank?

8. DR. SHANK: I have nothing to add.

9. DR. BERGFELD: Dr. Katz?

10. DR. KATZ: As part of your re-review, will you go back to the E.U. or go to the E.U. and ask them for their basis for their determination to see if you can obtain the data that they used when they determined that Disperse Blue 1 should not be used, that it was unsafe?

11. DR. ANDERSEN: Yes.

12. DR. BERGFELD: Dr. Belsito, is there a second?

13. DR. BELSITO: Second.

14. DR. BERGFELD: Second. Is there any further discussion then? Seeing none I'll call the question, all those in favor indicate by raising your hand. Thank you. Unanimous. We're going to reopen Disperse Blue.

15. Let's go on to the next ingredient, polyquaternium-7. Dr. Belsito?

16. DR. BELSITO: Yes. This safety assessment was published in 1995 with a safe-as-used conclusion. There were no significant new safety test data that would raise any concerns. However, the number of uses and the product categories of use have increased substantially including the potential for hair spray use. There is an additional wrinkle in that this is a polymer and one of the monomers is acrylamide and in the initial safety assessment of this it was acknowledged that acrylamide could be present at levels of up to 10 parts per million so that if you do the math, it's supplied at 8 percent used at 5 percent. The actual acrylamide monomer would be 0.04 parts per million which is significantly lower than the levels you would want. So we felt that there were really no new concerns here, that there was no need to reopen the document, that it was safe as used and that we that it's our assumption that it soon will be added, that a monograph is in development and it'll be added and used similar to the other ingredients in this report.

17. [Dr. Belsito] Okay, disperse Blue 1. And this is also a re-review. So the safety assessment in this was published in '95 with it a safe up to 1 percent. The basis for the 1 percent limit was not clear. There's no new data except that it's been considered by IARC and they now seem to be coming down on the side of possibly carcinogenic in humans. Certainly, when we looked at this in '95, we looked at carcinogenicity data very strongly and that's in our discussion. It should be noted that this is banned in Europe. Is that correct?

18. DR. EISENMANN: As far as we're aware of.

19. DR. BELSITO: So the question is do we
open it? We have -- in the supplemental report we have some additional information that -- from the FDA. And studies that were done on this, if you haven't had a chance to look at it, looking at absorption and comments from the FDA on industry's absorption data in terms of using frozen cadavers' skin, et cetera, it would increase absorption. But they still ended up changing a little bit industry's estimates. And coming up with two margin of safety estimates depending upon how you want to look at it. One where the margin was unsafe for potential carcinogenicity and the other where it was safe. And that had to do with whether you believed that this compound was renal toxic because it induced renal calculi. So it was a nongenotox mechanism and you applied a different factor for conservative estimates or whether it was genotoxic. So this was really way out of, I thought, my league in trying to decide whether to reopen. Reopen to ban it when it's not being used or we had already looked at the carcinogenicity and whether we feel that our assessment in the original report was okay.

So I'm going to turn this over to Curt and Dan and Paul for their comments because it's not a derm issue; it's a carcinogenicity issue.

DR. LIEBLER: Well, I had suggested in my notes here that we do not reopen. And that was mainly based on the lack of use.

DR. BELSITO: But if it's not used now and we said it could safely be used up to 1 percent and we decide not to reopen it, then I think what we're saying is that we have no concerns about it being used in the future at 1 percent. So I think if we have concerns then we have to reopen it. I mean, if we think it should not be used at all and we're not reopening it because it currently is not, I think we have to reopen it and ban it.

MS. FIUME: Because as of -- according to VCRP data as of February 18th of this year, it says there's three uses.

DR. EISENMANN: I think they're all old uses.

MS. FIUME: That was in response to an FOIA request. So it's dated as of February 18th.

DR. EISENMANN: I know, but --

DR. BELSITO: I don't think whether it's being used or not is the issue. I think if we don't reopen the report then we go on record at this point in time because we're looking at the data now. We go as of 2010 saying anyone who wants to use it up to 1 percent can safely use it up to that concentration. And if we think based upon the new data that we've seen or the rehashing of old data that that's not the case, then we need to reopen it to ban it if we don't think it should be used up to 1 percent. Or reopen it to say it's safe for use at a different concentration.

DR. SNYDER: I think we have to reopen.

What we do beyond that I think it will be driven by how we look at the data and how we want to incorporate the new data. And as you said, it could go two different directions. But I think we're obligated to reopen based upon the new data set that we received.

DR. BELSITO: Curt?

DR. KLAASSEN: Yeah, I think we need to look at this and think about this very seriously.

DR. BELSITO: Okay.

DR. LIEBLER: Fine. I'm fine with reopening. It sounds like we're all there.

DR. BELSITO: Okay. So we're going to reopen it to re-review the carcinogenicity data and any new carcinogenicity data that industry or anyone else wants to send us. And we're reopening it to determine whether we want to reaffirm our original conclusion, change the concentration limits, or possibly even ban it. We don't know yet.

DR. LIEBLER: I mean, it sounds like what we're reacting to is IARC's recent evaluation primarily.

DR. BELSITO: Right. And some comments we had gotten from the FDA after our -- or these
comments that the FDA had on the industry data that for some reason even though it was done in '94 I guess we didn't have available to us when the panel met. So this is their reevaluation of -- from some industry data we looked at. I think it would be very important if we can go to, you know, any information that was used by the SCCP and their decision on this if that's available as to why it was banned in Europe.

DR. EISENMANN: They just did not support it anymore.

DR. BELSITO: Right. So I think the data pieces we're going to need are going to be absorption.

DR. EISENMANN: There is an absorption from the FDA.

DR. BELSITO: I understand. Any additional information that may be out there. I'm just saying. You know, we have some absorption and we have FDA's comments about that absorption.

But if there is any additional information out there about the absorption of this molecule and any additional information on carcinogenicity that hasn't been -- and any additional information, you know, particularly looking at is this genotoxic or is it all because it's inducing, you know, calculi that are the cause for the tumors that we see.

And, you know, because based upon those different scenarios for causing cancer, you know, the FDA came up with different margins of safety for this.

Anything else? Okay. Hearing nothing we'll move on to the next one which is polyquat-7, also a re-review. In fact, the next tab.

So this was published in '95 as safe as used. There is no new safety data that's available. There is an additional wrinkle in that it seems that polyquaternium-7 is a polymer with one of the monomers being acrylamide. And in the original safety assessment of polyquat-7 it was acknowledged that acrylamide could be present at levels up to 10 parts per million. And the math was laid out. A combination of supplied at 8 percent used at 5 percent would equal 10 percent or 10 parts per million of the monomer would be in

DR. ANDERSEN: Thank you.

DR. BERGFELD: Reconsideration, I don't know, other terms.

DR. ANDERSEN: Yeah, but it -- I take your point.

DR. MARKS: Wilma? Any other singers?

DR. BERGFELD: That's it.

DR. MARKS: Okay. Thanks, Wilma. Those are important.

Now, we're on to Disperse Blue 1, if I'm correct? And that is in -- which book is that?

Buff 2.

Not reopen.

DR. SHANK: I agree.

DR. SLAGA: I agree.

DR. BERGFELD: How about unsafe?

DR. MARKS: Okay. Their decrease --

SPEAKER: There would be another alternative --


The big issue here, of course, is the carcinogenicity. That's correct. It's a mutagen and there is urine bladder cancer in rats, presumably somewhat related to the calculi that form in those rats, I guess. And based on my reading on absorption and negative dermatoxicity in mice, that we need not reopen this. That the conclusion safe for use in hair dyes at concentrations up to 1 percent is still valid.

And I guess we can take those points and put it in discussion points of why we didn't reopen and if we continue to use the term 'not reopen.' So we have -- does anybody feel other than Wilma that we shouldn't reopen it?

Okay.

DR. BERGFELD: I think you might discuss in -- obviously the discussion, that there's decreased use, continued surveillance of this dye, and then make our statement that we're basically on top of that.

Because, you know, we could easily call it unsafe at this time, I think.
DR. ANDERSEN: There is an issue that I guess I'd like to hear some discussion of. And that is the 1 percent limit. When I went back through and read the original safety assessment, I had a hard time figuring out what the basis for the 1 percent limit was. And if we're going to perpetuate that, it would be nice to know what the rationale is.

DR. HILL: It's right here in the --

COURT REPORTER: Microphone, please.

DR. HILL: Yeah, sorry. It's right here in the minutes, actually. Dr. McEwen stated --

DR. ANDERSEN: McEwen.

DR. HILL: McEwen? McEwen?

DR. ANDERSEN: McEwen.

DR. HILL: McEwen stated his preference for concentration of 1 percent and not .3 percent.

In his opinion, a 1 percent concentration limit could be justified based on the oral carcinogenicity study in which there was no effect in the animals tested.

Now, if that opinion translated into what actually came into the report, that's probably what it was.

DR. ANDERSEN: That wasn't captured in the discussion of the report.

DR. HILL: No, it was not.

MR. WILLIAMS: Maybe it should be now.

DR. SLAGA: Yeah, it should be put in, I think. And --

DR. ANDERSEN: We can do that.

DR. HILL: And also a negative dermal toxicity study -- carcinogenicity --

DR. ANDERSEN: Carcinogen --

DR. HILL: Carcinogenicity, excuse me.

DR. MARKS: Any other comments? So we'll catch that in the discussion, the issue of the carcinogenicity and the -- also the 1 percent level.

Not reopen? And then those will be (inaudible) 1 percent.

Okay. Any other comments about Disperse Blue 17? Final move -- motion will be not reopen.

Okay, next is polyquaternium-7. Again, same book.

They noted that these neoplasms appeared to be due to the formation of bladder calculi rather than a genotoxic mechanism. Such bladder calculi, while commonly seen in rats, do not appear to form in humans. Both biological and quantitative risk assessments demonstrated that the safe exposure concentrations reported in the NTP bioassay were significantly greater than the estimated maximum lifetime exposure associated with the use of semi-permanent hair dyes.

The Expert Panel also noted that these neoplasms were not found in dermal carcinogenicity studies in mice. Disperse Blue 1 also appears to be poorly absorbed in vivo studies. Based upon these data and due to the fact that exposure to hair dyes is brief, the Expert Panel concluded that Disperse Blue 1 is safe for use in hair dyes at concentrations up to 1% (End of Discussion).

The Panel voted unanimously in favor of issuing a Final Report on Disperse Blue 1 with the editorial changes/corrections that were requested.

Polyquaternium-7

Dr. Bergfield noted that comments were not received during the 60-day comment period for the Tentative Report that was announced. She also noted that the conclusion for this ingredient is safe as used.

Referring to the hematological assay results that are summarized in the section on short-term oral toxicity, Dr. Shank noted that whether or not the changes observed were statistically significant should be incorporated into the text.

In response to the conclusion that was expressed over different actual use concentrations of Polyquaternium 7 stated in the report text, Dr. McEwen noted that...
data exist, etc., this ingredient may be a potential human carcinogen under certain conditions.

Dr. Shank said that the oral carcinogenicity study was negative, the test was to agree with Dr. McEwen’s assessment. However, the oral carcinogenicity study was not negative; difficulty was experienced in interpreting these data. He also noted that Glutaral is highly reactive and that mutagenicity data was somewhat positive in several, but not all, of the tested systems. Furthermore, Glutaral was poorly absorbed in vivo and not poorly absorbed in vivo. Dr. Shank concluded that, in his opinion, these data do not support the safety of an ingredient in leave-on products.

The Panel concluded that Glutaral is not used in rinse-off products and that there is insufficient data to confirm its safety in leave-on products. With the exception of Dr. Carlson, all Panel members voted in favor of this conclusion.

Dr. Bergfeld stated that the report discussion would have to be revised such that the Panel’s concerns regarding the absorption, reactivity, and genotoxicity of Glutaral, and the need for an NTP 2-year dermal carcinogenicity study on this ingredient are detailed.

Dr. Carlson noted that it should be stated in the discussion that the 2-year dermal carcinogenicity study should not be conducted using Fisher-344 rats.

**DISPERSE BLUE 1**

Dr. Schreiner noted that Disperse Blue 1 is used as a colorant in hair dyes, and that cutaneous absorption data and any assessment of carcinogenic potential were requested. Furthermore, it was determined that if these data are not considered adequate, a 2-year NTP dermal carcinogenicity study should be performed.

Dr. Beltsio recalled that the dermal absorption data had been received, and that absorption did not exceed 0.15% after 3 h.

Based on the absorption data, the Beltsio and Schreiner Teams concluded that Disperse Blue 1 is safe at concentrations up to 1%.

Dr. Bergfeld asked if the report discussion which addresses the administration of small doses of Disperse Blue 1 in toxicity studies, calcification formation in the urinary tract of rats, and the erratic mutagenicity data (positive and negative effects) should be developed.

Dr. Andersen said that it, in the animal model, calcification is part of the mechanism for toxicity, then this observation has limited relevance to the human experience because the formation of calcification is not likely. He agreed that this issue should be mentioned in the report discussion.

Dr. Stoa said that the large number of positive mutagenicity studies on Disperse Blue 1 that are available should be mentioned in the discussion, such that the public knows that the Panel is aware of these findings.

Dr. Shank noted that Disperse Blue 1 is used in rinse-off products and that it is very poorly and slowly absorbed. Therefore, the level of exposure is very low. These statements were also recommended for inclusion in the report discussion. This information also provides justification for the administration of small doses of Disperse Blue 1 in toxicity studies that were performed.

Concerning the administration of small doses of Disperse Blue 1 in toxicity tests, Dr. McEwen noted that it would have to be acknowledged in the discussion that these doses are representative of many times the exposure that human subjects would be subjected to under normal use conditions.

The Panel unanimously concluded that Disperse Blue 1 is safe for use in cosmetics at concentrations up to 1%.

**PYRACATECHOL**

Dr. Beltsio noted that Pyracatechol has been banned in Europe. However, he said that none of the available data that have been included in the report addendum, it is not possible to determine whether or not this ingredient is safe for use in cosmetics. With this in mind, the Panel requested the following data from industry at the November 22-23, 1993 Panel meeting: (1) The extent and speed of the oxidative reaction in hair dyeing. If Pyracatechol is not rapidly and completely oxidized, the Panel recommend that the following additional data be requested: (2) Cutaneous absorption (aqueous or alcohol vehicle), (3) Method of manufacture, (4) Impurities, and (5) UV absorption data; if Pyracatechol absorbs in the UVA or UVB range, photosensitization studies will be required. To date, none of the data requested by the Panel have been received.

Dr. McEwen noted that, in many cases, the method of manufacture is held as proprietary information by the company and asked whether the Panel really needed these data.

The Panel agreed that the request for method of manufacture could be delayed and that impurities data would be sufficient.

The Panel unanimously voted in favor of issuing an insufficient data announcement.

**DISPERSE BLUE 1**

The Panel voted in favor of adding an insufficient data announcement on Disperse Blue 1 Adipate. The four items that are listed immediately above will be included in this announcement.

Dr. Bergfeld noted that Disperse Blue 1, a hair dye and that this was the first time that the report on this ingredient had been reviewed by the full Panel.

Dr. Beltsio said that his Team determined that Disperse Blue 1 is safe as used in hair dyes at concentrations up to 1%. He also noted that the revised hair dye epidemiology database would be incorporated into the COR report.

Dr. Bergfeld asked if there was any reason for concern about the carcinogenicity of Disperse Blue 1.

Dr. Shank noted that Disperse Blue 1 is used up to a concentration of 1% in hair dyes, and that the ingredient was tested at a concentration of 0.1 or 0.2% (very low doses) in a carcinogenicity study.

Dr. Beltsio noted that carcinomas were observed in rats and that the results were very questionable in mice.

Dr. Shank thought that the concentration limit should be 0.3%, based on the results of a dermal carcinogenicity study. However, he noted that the investigators’ conclusions regarding the carcinogenicity of Disperse Blue 1 are not stated in the text of the COR report, and that this information is needed, particularly in that it is stated in the text that compounds that are structurally related to this ingredient are carcinogenic.
Dr. Klaasen stated that based on the available studies, Disperse Blue 1 is a carcinogen in male and female rats when administered orally, but is not carcinogenic when administered orally to male or female mice. Furthermore, the negative dermal carcinogenicity data on Disperse Blue 1 is from a study involving mice. He then asked how much confidence should be placed in a mouse dermal carcinogenicity study, considering that Disperse Blue 1 is not carcinogenic when administered orally to mice.

Dr. Sieg wanted to know if the carcinogenicity studies summarized in the CIR report could be made available for review by the toxicologists on the Panel.

Dr. Andersen indicated that the reports could be made available for review during the meeting.

Dr. McEwen stated his preference for a concentration limit of 1% and not 0.3%. In his opinion, a 1% concentration limit could be justified based on the oral carcinogenicity study in which there was no effect in the animals tested. Dr. McEwen suggested that the Panel review the NTP oral feeding study and determine what the administered dose would be relative to dermal application.

After reviewing the 1985 NTP oral feeding study, Dr. Schroeter's Team concluded that Disperse Blue 1 is carcinogenic when administered orally and that dermal absorption data are needed.

Dr. Andersen wanted to know why a dermal absorption study is needed, considering that a negative dermal carcinogenicity study is included in the CIR report.

Dr. Shank noted that the data included in the negative dermal carcinogenicity study (Jacobs et al., 1984) are not sufficient, because a formulation containing more than one hair dye was tested in this study. Data on individual compounds are not included.

Dr. McEwen said that data on the skin penetration of Disperse Blue 1 and more details from the dermal carcinogenicity study could be supplied.

Dr. Belsito noted that the fact that Disperse Blue 1 was carcinogenic in rats, but not in mice, in oral feeding studies is one of the concerns that had been expressed by the Panel. He also said that the question relating to which of the two species is relevant to humans in terms of oral carcinogenicity will have to be addressed.

Dr. Corbett noted that a biological risk assessment, based on the NTP rat oral feeding study and a mathematical risk assessment, based on skin absorption data, are available and can be submitted to the Panel. He also stated that risk assessments have not been submitted to the Panel in the past because industry was under the impression that the Panel did not use quantitative risk assessments in safety evaluations.

The Panel voted in favor of tabling the report on Disperse Blue 1 until the February 26 - March 1, 1984 Expert Panel meeting, with the understanding that the following information will be available for review prior to that meeting: (1) Skin penetration data (human skin); (2) Biological and mathematical risk assessments that were promised by Dr. Corbett; and (3) Any additional details from the dermal carcinogenicity study by Jacobs et al., 1984.

UROCANIC ACID

Dr. Bergfeld noted that Urocanic Acid is a Wyeth ingredient that has been under review by the Panel for several years. The main issues that have been raised in

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Draft Amended Report

Disperse Blue 1 as used in Cosmetics

November 18, 2010
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INTRODUCTION

Disperse blue 1 was previously reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel. In 1995, the safety assessment was published with the conclusion that disperse blue 1 is “safe for use in hair dyes at concentrations up to 1%.”

In 2005, the National Toxicology Program (NTP) 11th Report on Carcinogens stated that disperse blue 1 is “reasonably anticipated to be a human carcinogen.” An excerpt from that report is included in this re-review. The International Agency for Research on Cancer (IARC) had previously concluded in 1990 that disperse blue 1 is “possibly carcinogenic to humans.” Although the IARC report was issued in 1990, the IARC conclusion was not included in the original CIR safety assessment. In addition, an unpublished Food and Drug Administration evaluation of the carcinogenic potential of Disperse Blue 1 is newly available.

The original assessment included carcinogenicity data as well as risk assessments addressing those carcinogenicity data. That original information is included in this re-review document so that the Panel has ready access to all information.

A search of the available recent literature identified an in vitro dermal absorption study and case studies regarding contact allergy. Updated frequency of use data indicate that inclusion of disperse blue 1 in hair dye formulations has declined to near-zero. It has recently been reported to be used in only 3 formulations, compared to 112 at the time of the original assessment. Industry reports no use in response to the recent survey undertaken by the Personal Care Products Council (Council).

Summary information from the original report is included at the beginning of each section. Little new data were available, but the new data includes dermal penetration data and the risk assessment data that were not included in the original report.

While the Expert Panel discussed the cancer risk data during the original review of disperse blue 1, rejecting potential genotoxic mechanisms of action, this safety assessment was reopened to examine all new data. When available, the new information is also summarized at the beginning of each section and is italicized.

CHEMISTRY

Definition and Structure

Disperse blue 1 (CAS No. 2475-45-8) is classified chemically as an anthraquinone color. It has a log P of -1.65. The structure of disperse blue 1 is presented in Figure 1, and synonyms are given in Table 1.

Impurities

Commercial preparations of disperse blue 1 are approximately 50% disperse blue 1, 30% structurally-related compounds, and 20% water. These preparations are approximately equal amounts of dyestuff and lignosulfonate dispersants. Two major impurities of disperse blue 1 are an isomer of the dye and triamino-nitroanthraquinone, at concentrations of approximately 25 and 6%, respectively.


USE

Cosmetic

Disperse blue 1 is reported to function in cosmetic formulations as a hair colorant. Table 2 presents the historical and current product formulation data for disperse blue 1. In 1994, according to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP), disperse blue 1 was used in 112 hair color formulations. Industry reported that in 1994, disperse blue 1 was used at a concentration of 0.62% in semi-permanent hair dyes and that it was not used in conjunction with hydrogen peroxide. There are no current uses for Disperse
Blue 1 in the VCRP (personal communication, Donald Havery). This report of 0 use is in agreement with the results of a 2009 industry concentration of use survey conducted by the Council, in which no uses were reported.\textsuperscript{5}

Disperse blue 1 is listed in Annex II of the European Union Cosmetics Directive, which is the list of substances which must not form part of the composition of cosmetic products.\textsuperscript{8} The basis for disperse blue 1 not being allowed in cosmetics in Europe is that it is listed in Annex 1 of the Dangerous Substances Directive as a category 2 CMR (carcinogenic, mutagenic, or toxic to reproduction) compound with R45 (carcinogen) designation.\textsuperscript{9} Category 2 carcinogens are defined as “substances which should be regarded as if they are carcinogenic to man.” Disperse blue 1 is on Health Canada’s list of high health priorities for action for substances which are lowest potential for exposure and high hazard substances.\textsuperscript{10}

**GENERAL BIOLOGY**

**Dermal Absorption**

The 1995 safety assessment concluded that disperse blue 1 was poorly absorbed through the skin. The dermal penetration of a formulation containing 1% disperse blue 1, tested in vitro using skin from miniature pigs, did not exceed 0.15% of the dose.

From the Final Report on the Safety Assessment of Disperse Blue 1.\textsuperscript{1}

*Current data indicate that 0.2% of the dose of disperse blue 1 applied in vitro to human skin was found in the receptor fluid. This was consistent with both an ethanol and a spiked semi-permanent vehicle. Using rat skin, 0.4-0.7% of the dose was found in the receptor fluid. The vehicle affected the amount of dose that remained in the skin, with more disperse blue 1 being found in the skin when an ethanol vehicle was used, as compared to the semi-permanent vehicle. Little of the disperse blue 1 that penetrated the skin within 24 h moved into the receptor fluid over 72 h.*

The in vitro penetration of disperse blue 1 through human abdominal skin and female fuzzy rat skin was determined using flow-through diffusion cells.\textsuperscript{7} A semi-permanent formulation containing disperse blue 1, which consisted of a representative dye base containing disperse blue 1 and 14C-disperse blue 1, was evaluated in both a pure ethanol and a spiked semi-permanent hair color formulation. The total disperse blue 1 content (non- and radiolabeled) was 0.52% in the formulation. Approximately 2 µg of disperse blue 1 was applied to the skin in each diffusion cell. All dosing vehicles were applied to the skin for 30 min. The amount of disperse blue 1 remaining in the skin was determined after 24 or 72 h.

Disperse blue 1 “slowly penetrated human skin with relatively constant absorption over 24 h”. In human skin (n=4), absorption into the receptor fluid over 24 h, with both the ethanol and semi-permanent vehicle, was 0.2%. Although absorption into the receptor fluid did not vary much with vehicle, approximately 11% of the dose remained in the skin with the ethanol vehicle compared to only 3% with the semi-permanent vehicle, indicating that total penetration of disperse blue 1 into human skin was significantly lower with the semi-permanent hair dye vehicle compared to ethanol. The researchers stated that disperse blue 1 may have been more soluble in the semi-permanent vehicle, and this could have affected partition in the skin.

Relatively constant absorption was again seen using fuzzy rat skin (n=3) during the 24 h period; 0.7 and 0.4% of the applied dose absorbed into the receptor fluid with the ethanol and semi-permanent vehicles, respectively. As with human skin, that total penetration of disperse blue 1 into fuzzy rat skin was significantly lower with the semi-permanent hair dye vehicle compared to ethanol, as approximately 9 and 2.6% of the dose remained in the skin with the ethanol and semi-permanent vehicle, respectively. The majority of disperse blue 1 (approximately 80%) was found in the stratum corneum, while the remainder was in the viable epidermis/dermis layer.

The researchers then examined the fate of disperse blue 1 in the rat skin reservoir using a 72 h absorption study in which the skin was rinsed after 24 h. Only 0.16% of the applied dose of disperse blue 1 that penetrated the skin within 24 h
(i.e., 2.6% of the applied dose) moved through the skin into the receptor fluid over 72 h. (It appears that the semi-permanent vehicle was used.) It was concluded that the skin content should not be regarded as absorbed material, and that the amount of disperse blue 1 systemically available was the amount that moved into the receptor fluid at 24 h. No skin metabolism of disperse blue 1 was noted upon absorption, as indicated by the presence of only a single peak for disperse blue 1 on a high performance liquid chromatography chromatogram.

**ANIMAL TOXICOLOGY**

The oral LD$_{50}$ of disperse blue 1 in corn oil was $>3000$ mg/kg for rats and $>2000$ mg/kg for mice. In a study in which rats were dosed with 1 g/kg disperse blue 1 by gavage for 1-3 days, dye was found in the kidneys of rats dosed by gavage, leading to hyperplasia of the renal pelvis epithelium, and nephropathy was observed. For the rats fed 1% disperse blue 1 in feed for 4 days, there was no accumulation in the kidneys, but there was low-grade hyperplasia of the bladder urothelium. In a 14-day dietary study with $\leq 50,000$ ppm disperse blue 1, some mid-dose groups of rats and mice had decreased body weight gains, the high-dose rats and mid-dose mice were inactive, and all test animals excreted blue urine and had blue organs at necropsy. In a 13-wk dietary study in which rats were fed $\leq 20,000$ ppm and mice were fed $\leq 10,000$ ppm disperse blue 1, the higher dose group rats and mice had decreased final body weights, all test animals excreted blue urine, and male and female rats and mice fed diets containing $\geq 2500$ ppm had pigmentation of the thyroid gland follicle, renal pigmentation, lesions in the urinary bladder, and urinary tract calculi, and mice had nephrosis and focal myocardial necrosis. In a 2-yr study, no adverse effects were observed when dogs were fed 19.5 or 97.5 mg/kg of a formulation containing 1.54% disperse blue 1.

From the Final Report on the Safety Assessment of Disperse Blue 1.$^1$

**Irritation/Sensitization**

The irritation threshold for disperse blue 1 was $> 10\%$, and 1% disperse blue 1 was a moderate sensitizer in studies with guinea pigs.

From the Final Report on the Safety Assessment of Disperse Blue 1.$^1$

A local lymph node assay (LLNA) was performed using female BALB/c mice to determine the sensitization potential of disperse blue 1.$^{11}$ Fifty µl of a 3% and a 10% solution of disperse blue 1 in dimethyl sulfoxide was applied once daily to a 2 cm$^2$ shaved area on the back of 7-10 mice on days 1-3 of the study. Following 10 days of non-treatment, 25 µl of the test solution was applied to the dorsum of both ears on day 15-17 of the study. The animals were killed on day 19, at which time the lymph nodes were prepared and various endpoints were analyzed. A negative control group of 20 animals was treated with vehicle only. Lymph node weights were significantly increased with 3 and 10% disperse blue 1, while significant increases in ear thickness and ear punch weight were observed only with the 3% solution. Disperse blue 1, 3%, was classified as a moderate sensitizer in this LLNA.

**REPRODUCTIVE AND DEVELOPMENTAL TOXICITY**

No adverse reproductive or teratogenic effects were observed in feed or gavage studies using rats and/or rabbits of dye formulations containing 1.54% disperse blue 1.

From the Final Report on the Safety Assessment of Disperse Blue 1.$^1$

**GENOTOXICITY**

In Ames tests, disperse blue 1 was mutagenic to some strains of Salmonella typhimurium, but conflicting results were sometimes obtained between studies. Positive results were also obtained in an L5178Y mouse lymphoma cell mutation assay (concentrations of $\leq 160$ µg/ml), a chromosome aberration test using Chinese hamster ovary (CHO) cells (at concentrations of $\geq 7.5$ µg/ml with metabolic activation and $\geq 9$ µg/kg without), and a sister chromatid exchange test (at concentrations $\geq 3.3$ µg/ml without metabolic activation). A semi-permanent hair dye containing 0.12% disperse blue 1 was evaluated in an in vivo heritable translocation study, and no effects were seen.

From the Final Report on the Safety Assessment of Disperse Blue 1.$^1$
**CARCINOGENICITY**

In a carcinogenicity bioassay in which rats and mice were fed diets containing ≤5000 ppm and ≤2500 ppm disperse blue 1, respectively, disperse blue 1 was carcinogenic in male and female rats. It increased the incidence of transitional cell papillomas and carcinomas, of leiomyomas and leiomyosarcomas, and of squamous cell papillomas and carcinomas of the urinary bladder. Equivocal results were obtained in tests with male mice; neoplastic lesions included hepatocellular adenomas or carcinomas (combined) in low and mid-dose males and alveolar/bronchiolar adenomas or carcinomas (combined) in high dose males. Negative results were reported in female mice. In another dietary study, rats were given diets containing ≤0.1% disperse blue 1 for 19 mos or 1.0% for 6 mos, the rats fed 1.0% developed lesions of the urinary bladder and kidneys, which were associated with dye deposits and calculi. However, no sarcomas of the bladder wall or epithelial neoplasms were observed. Results of a dermal carcinogenicity study, evaluating two non-oxidative hair dye formulations containing 0.1 or 0.3% disperse blue 1 were negative. Three compounds that are structurally-related to disperse blue 1, i.e. 2-aminoanthraquinone, 1-amino-2-methyl-anthraquinone, and 2-methyl-1-nitroantraquione, were carcinogenic to rats and mice in feeding studies. Two of the chemicals affected the liver, while the third affected the stomach and bladder.

From the Final Report on the Safety Assessment of Disperse Blue 1.1

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Actual text from the Final Report on the Safety Assessment of Disperse Blue 1

A commercial grade of Disperse Blue 1 (minus lignosulfonate dispersants) was tested for carcinogenic potential by the NTP (1986). Groups of 50 male and 50 female F344/N rats were fed diets containing 1,250, 2,500, or 5,000 ppm Disperse Blue 1, and groups of 50 male and 50 female B6C3F1 mice were fed diets containing 600, 1,200, or 2,500 ppm Disperse Blue 1 for 2 years. Control groups of animals were fed an untreated diet. The animals were observed twice a day, and clinical signs were recorded once per week. Body weights were monitored weekly for the first 13 weeks and monthly thereafter. Necropsy was performed on all of the animals either at the time of death or when they were killed at the end of the study.

The daily consumption of Disperse Blue 1 for the low, mid, and high dosage rats were: 45, 95, and 217 mg/kg, respectively, for the males, and 56, 111, and 240 mg/kg, respectively, for the females. Throughout the study, the mean body weights of the male and female rats in the high dosage group were lower than that of the control groups, and the rats from the mid dosage group had marginally lower body weights than the controls. The survival rates of the high dosage male and female rats were significantly reduced after 65 and 72 weeks, respectively. The males from the mid dosage group had marginally reduced survival rates, until week 100 when the rate became significantly reduced. Clinical signs of toxicity observed during the study included blue urine, firmness in the area of the urinary bladder, wet fur in the pelvic area, blue fur and extremities, and feces stained blue-green. Some of the female rats fed the high dosage had blue crusty material in the vaginal area.

The most significant pathologic changes were found in the urinary system of both the male and female rats. There was an increased incidence of the following nonneoplastic lesions: renal and urinary bladder calculi, renal casts, hydroephrosis and renal degeneration, renal and urinary bladder epithelial hyperplasia, urinary bladder squamous metaplasia, and pigmentation of the kidneys and urinary bladder. A number of different tumor types were also found in the urinary bladder. There was a dosage-related incidence of the following neoplasms: transitional cell neoplasms and leiomyosarcomas in the male rats of the mid and high dosage groups; squamous cell neoplasms in the males of the high dosage group; transitional cell papillomas and transitional cell carcinomas in the females of the mid and high dosage groups; and squamous cell papillomas, squamous cell carcinomas, leiomyoma, and leiomyosarcoma in the females fed the high dosage. Lipomatosis of the urinary bladder was also found in nine mid dosage female rats and one high dosage female rat; but it could not be determined if these lesions were neoplastic.
The investigators noted that urinary bladder calculi were often found in the rats with bladder neoplasms, which suggested that the calculi may have influenced the occurrence of the neoplasms in the rats. However, a number of rats had urinary bladder neoplasms without observed calculi. There was also a marginally increased incidence of pancreatic islet cell adenomas or carcinomas (combined) in male rats treated with Disperse Blue 1. Based upon these observations, the conclusions were that there was “clear evidence of carcinogenicity for male and female F344/N rats as shown by the increased occurrence of transitional cell papillomas and carcinomas, of leiomyomas and leiomyosarcomas, and of squamous cell papillomas and carcinomas of the urinary bladder.”

In the study with the B6C3F mice, the daily consumption of Disperse Blue 1 for the low, mid, and high dosage animals was calculated to be 112, 239, and 540 mg/kg per day for the males, and 108, 235, and 520 mg/kg per day for the females.

The mean body weight of the females fed the high dosage were generally lower than that of the female controls, while the females of the low dosage group had generally greater body weights. The mean body weights of the male mice were comparable to that of the controls. With the male mice, there was a significant trend toward lower survival with increasing doses of Disperse Blue 1; however, none of the dosage groups had a significant reduction in survival in pairwise comparisons with controls. There was no significant difference in survival between the groups of female mice and the controls. Treatment-related clinical signs of toxicity in the male rats included alopecia, externally cannibalized genitalia, and scratches. Both male and female mice had blue fur, blue urine, and firmness in the area of the urinary bladder.

Non-neoplastic lesions found at significantly increased incidences in the urinary system of both the male and female mice were blue pigmentation of the urinary bladder and kidneys, inflammation and epithelial hyperplasia in the urinary bladder, calculi in the urinary bladder lumen, fibrosis of the urinary bladder, casts in the renal tubular lumina, and renal tubular degeneration. There were no significant increases in the number of neoplastic lesions found in the urinary bladder or kidneys. Neoplastic lesions found at increased incidences elsewhere in the body were hepatocellular adenomas in the females of the low dosage group, hepatocellular adenomas or carcinomas (combined) in the low and mid dosage male mice, and alveolar/bronchiolar adenomas or carcinomas (combined) in the high dosage male mice. Based on these findings, the authors concluded that there was “equivocal evidence of carcinogenicity of [Disperse Blue I] in male B6C3F1”, and that there was “no evidence of carcinogenicity of [Disperse Blue I] in female B6C3F1 mice”.

Burnett and Squire (1986) conducted chronic studies to determine the effect of dietary administration of Disperse Blue 1 on the urinary system of Fischer 344 rats. Groups of 20 male and 20 female rats were fed a diet containing 0.01 or 0.10% Disperse Blue 1 for 19 months. Another group of 60 male and 60 female rats were administered a diet of 1.0% Disperse Blue 1 for 6 months. A control group of 40 male and 40 female rats were fed untreated diet. Body weights and feed consumption were recorded weekly for the first 3 months, and then monthly thereafter. These parameters were reduced only in the 1.0% Disperse Blue 1 group, and were rarely <90% of the controls. Urine samples were collected after 4, 8, 18, 23, and 35 weeks for pH determination. A few statistically significant different pH values were found, but the incidence of these changes were scattered. The urine of all the treated animals was blue.

After 5, 9, and 17 weeks on the diets, 3-5 rats were killed from each of the groups for necropsy. Multiple small dark blue particles and sediment were found in many of the urinary bladders from the rats fed the 1.0% Disperse Blue 1 diet. These particles increased in size with time. With one exception, all of the rats with tumors had one or more large calculi. The calculi ranged in weight from 0.013 to 0.402 g in male rats, were smooth and very dark blue, and consisted mainly of Disperse Blue 1. The calculi found in the female rats ranged in weight from 0.687 to 6.170 g, were generally larger than
those found in the male rats, were rough, blue-brown and occasionally mottled in appearance, and were composed of mostly calcium phosphate.

The results of autoradiographic examination of the urinary bladder were extensive labeling in the transitional epithelial cell nuclei of the rats fed the 1% Disperse Blue 1 diet. The extent of labeling was correlated with the extent of simple or papillary hyperplasia. Hyperplasia was slight to moderate at week 5, and metaplasia was present at weeks 9 and 17. Several of the animals also had clumps of dye present on the epithelium. One male rat had a transitional cell papilloma and one female rat had a squamous cell papilloma. No increase in nuclear labeling and no lesions were found in the bladders of the animals from the 0.01 and 0.1% Disperse Blue 1 treatment groups.

Changes in the kidneys were observed only in the animals fed the 1.0% Disperse Blue 1 diet for 17 weeks. The severity of these changes correlated with the amount of dye present in the renal tissues and the length of treatment. Tubular degeneration and regeneration with interstitial fibrosis and inflammation were the most common lesion observed. The tubular detail was often distorted by dye present within the tubules. Hyperplasia of pelvis epithelium and dye particles in or on the pelvis epithelium were also observed. No dye or treatment-related changes were found in the kidneys from the rats fed the 0.01 and 0.1% Disperse Blue 1 diets for as long as 9 weeks.

After 6 months, 10 male and 10 female rats fed the 1.0% Disperse Blue 1 diet and 12 male and 12 female rats fed the control diet were killed and their urinary bladders and kidneys were examined. The urinary bladders of most of the male rats contained calculi weighing up to 400 mg. Fewer calculi were found in the urinary bladders of the female rats, but fine dark blue sediment was often present. Moderate epithelial hyperplasia of the bladder was found in all of the rats, and squamous metaplasia was present in most of the animals. Other changes included dye on or beneath the epithelium, two rats with squamous cell papilloma, and one rat with transitional cell carcinoma. There was also focal accumulation of histiocytes beneath the bladder epithelium in association with dye particles in two of the rats. In the kidneys, Disperse Blue 1 was found in the tubules of all of the rats and pelvis epithelial hyperplasia was present. Some of the animals also had squamous metaplasia of the pelvis epithelium.

The remaining animals from the 1.0% Disperse Blue 1 group were fed the control diet for an additional 13 months and the calculi from the urinary bladders of 15 male and 15 female rats were surgically removed after 2 weeks on the control diet. The object of this procedure was to determine whether, without further exposure to the dye, the persisting foreign material could influence carcinogenesis. The number of bladder calculi and the development of severe bladder lesions were much greater in the female rats that had surgery than those that did not. The authors concluded that surgery stimulated the formation of even more calculi in the female rats. In the male rats, calculus development was minimal in those that underwent surgery and the degree of hyperplasia-metaplasia was decreased. In the males not receiving surgery, one transitional cell papilloma was found and most of the animals had minimal hyperplasia. The urinary bladder and kidneys of the animals maintained continuously on the 0.01 and 0.1% Disperse Blue 1 diets for 19 months were comparable to those of the control animals.

Two non-oxidative hair dye formulations containing 0.10 and 0.3% Disperse Blue 1 were tested for carcinogenic potential using Swiss Webster mice. Each formulation was applied to the clipped interscapular region of 60 male and 60 female mice three times a week for 20 months. Two control groups had the same shaving schedule but were left untreated. The mice were observed regularly for signs of toxicity. Ten mice of each sex were killed from each group after 9 months for clinical tests, hematology profiles, and necropsy. Necropsy was performed on the remaining animals either at the time of interim death or when they were killed at the end of the study.
No adverse effects on body weight gains or survival were observed in either of the treatment groups, and there was no evidence of toxicity from the hematological or urinary values. Some of the animals had chronic inflammation of the skin, but this was also observed in the control animals. Hemangiomas of the liver, adenomas of the lungs, and malignant lymphomas were observed in some of the treated animals, but the incidences of these neoplasms were not significantly different from those observed in the two control groups. The authors noted that these lesions are commonly found in Swiss Webster mice and concluded that no carcinogenic effects were clearly indicated (Jacobs et al., 1984).

**CLINICAL ASSESSMENT OF SAFETY**

**Irritation/Sensitization – Case Studies**

In the 1995 assessment, it was reported that 1 of 15 patients with contact dermatitis from textile dyes had a positive patch-test reaction to disperse blue 1 (concentration not specified).


Currently, one case study in which reactions to black “velvet” clothes were reported, strong reactions to disperse blue 1 were not observed.

Disperse blue 1 has been identified as a textile dye contact allergen. Case studies regarding black “velvet” clothes have been reported. In 9 cases in which reactions have been reported, patch testing was performed with dyes to determine the cause of the reaction. Disperse blue 1 in 1% white petrolatum resulted in no reactions at day 1 and a “+” reaction for one and a “++” reaction for 2 of the 9 subjects. The subjects always had strong reactions to some other disperse blue dyes. It was determined that “low amounts” of disperse blue 1 were used in these fabrics.

**Carcinogenic Potential**

It is stated that the NTP found that disperse blue 1 is reasonably anticipated to be a human carcinogen, and the IARC found that there was sufficient evidence of carcinogenicity in animals to conclude that disperse blue 1 is possibly carcinogenic to humans.

In its 11th Report on Carcinogens, the NTP stated that disperse blue 1 is “reasonably anticipated to be a human carcinogen” based on evidence of malignant tumor formation in experimental animals to an unusual degree with regard to incidence, site, and type of tumor (NTP 1986), and because it is an anthraquinone and, therefore, structurally related to other substances listed in a previous Annual Report on Carcinogens as either known to be human carcinogens or reasonably anticipated to be human carcinogens (NTP 1994),” It was noted that the tumors induced in the urinary bladder of rats by disperse blue 1 would probably not occur in humans, but the report states: “However, compelling data that demonstrate a causal relationship between urinary bladder calculi and leiomyomas and leiomyosarcomas have not been sufficiently developed to contradict other evidence that Disperse Blue 1 is reasonably anticipated to be a human carcinogen.”

According to the IARC, there was sufficient evidence for the carcinogenicity of disperse blue 1 in experimental animals for an overall evaluation that “Disperse Blue 1 is possibly carcinogenic to humans (Group 2B).”

**EPIDEMIOLOGY**

Hair dyes may be broadly grouped into oxidative (permanent) and direct (semi-permanent) hair dyes. The oxidative dyes consist of precursors mixed with developers to produce color, while direct hair dyes are a preformed color. Disperse blue 1 is a direct hair dye ingredient. While the safety of individual hair dye ingredients are not addressed in epidemiology studies that seek to determine links, if any, between hair dye use and disease, such studies do provide broad information. The CIR Expert Panel noted the conclusions of these reviews, including that personal use of hair colorants, cannot be evaluated as to its carcinogenicity and that occupation as a hairdresser or barber entails exposures that are probably carcinogenic, insufficient evidence exists to support a causal association between personal hair dye use and a variety of tumors and cancers such
as acute leukemia, bladder cancer, multiple myeloma, and non-Hodgkin’s lymphoma,\textsuperscript{20} and the epidemiological evidence for personal use of hair colorants is inadequate and is not classifiable as to its carcinogenicity to humans.\textsuperscript{21} The following ‘Risk Assessment’ section addresses disperse blue 1 specifically.

**Risk Assessment**

The 1995 safety assessment stated that risk assessments using both biologic and quantitative approaches indicate that hair dye use of disperse blue 1 does not pose a carcinogenic risk to humans. Safe exposure concentrations determined in the NTP bioassay were significantly greater than the estimated maximum lifetime average daily applied dose associated with the use of semi-permanent hair dyes. Both risk assessments indicated the belief that disperse blue 1 was not acting through a direct genotoxic mechanism, and that any weak mutagenic activity that occurred could be due to mutagenic contaminants.

*From the Final Report on the Safety Assessment of Disperse Blue 1.*\textsuperscript{1}

Updated risk assessment information indicates that the maximum absorbed-dose rate of disperse blue 1 for lifetime hair-dye use would be 0.44 µg/kg/day, assuming that all of the product applied to the hair contacts the scalp, and 0.2% of that penetrates the skin. This value (0.44 µg/kg/day) is slightly greater than the oral dose-rate estimated to be associated with a 1:1,000,000 upper-bound lifetime cancer risk, based on a simple linear-regression analysis of bladder tumor data from rats, which was 0.3 µg/kg/day. An estimated acceptable daily intake, assuming a non-genotoxic mechanism of action, is 100-times greater than the maximum absorbed-dose rate estimated for lifetime hair-dye use. Quantitative estimates of the risks associated with the use of disperse blue 1 depend on whether it is viewed as a genotoxic or non-genotoxic agent. However, current conservative risk estimates suggest that the use of disperse blue 1 in hair-dye formulations would be associated with no significant cancer risks, regardless of the mechanisms of action.

**Risk Assessment Text from the Original Safety Assessment\textsuperscript{1}**

Reviewing the data of the NTP (1986)\textsuperscript{12} and Burnett and Squire (1986)\textsuperscript{13} studies, Couture-Haws et al. (1994) cited the weight of the evidence for a secondary carcinogenic mechanism of action for this ingredient.\textsuperscript{22} Specifically, they believed that Disperse Blue 1 acted through a threshold mechanism involving the formation of urinary calculi to induce bladder tumors in rats. They noted that not only was the occurrence of bladder neoplasms associated with the occurrence of bladder calculi, but that no-observed-adverse-effect levels (NOAELs) were determined, there was a correlation between the degree of epithelial hyperplasia and the induction of urinary bladder neoplasms, and the presence of dye particles in subepithelial layers was associated with the tumors of mesenchymal origin. They noted that bladder neoplasms occurred in both male and female rats, regardless of differences in chemical composition of the urinary bladder calculi, and that progression of urinary bladder tumors was halted in rats when Disperse Blue 1 treatment was discontinued. The authors believed this indicated that Disperse Blue 1 as not acting through a direct genotoxic mechanism.

They acknowledged that there was limited and weak evidence of mutagenicity in in vitro studies. However, it was noted there were no strong positive results obtained even at the highest concentrations tested. Additionally, there was a lack of dose-response, there were questions regarding solubility, and there was a possibility that mutagenic contaminants were present.

The authors evaluated the human safety of Disperse Blue 1 using two types of risk assessments: a biologically based approach and a conventional quantitative approach. In the first approach, an uncertainty factor of 1,000 was applied to the NOAEL in the NTP bioassay, which indicated a safe exposure level of 45-56 µg/kg per day. This value is 20 times greater than the estimated maximum lifetime average daily applied dose associated with use of semi-permanent hair dyes (2.7 µg/kg per day). In the second approach, the linearized multistage model was applied to data on the incidence of leiomyomas and leiomyosarcomas. An exposure level corresponding to an upper limit of lifetime risk of $10^{-6}$ or $10^{-5}$ was 0.39 or 3.9 µg/kg per day, respectively. The latter value is 1.5 times greater than the estimated maximum lifetime exposure from semi-permanent
hair dye use. The authors concluded, “Because oral absorption is substantially more than dermal absorption, the actual margin of safety is most likely much greater than either of these comparisons suggest”.

A similar conclusion was also reached in a separate evaluation by Weisburger (1989). Using the Decision Point Approach, it was noted that in the NTP (1986) bioassay Disperse Blue 1 could not be a carcinogen of the same types as 2-acetylaminofluorene or 4-aminobiphenyl or aflatoxin B1. The effects on the kidneys and urinary bladder of rats were not observed in the study with mice. Mutagenicity test results indicated a weak mutagenic response, but the author stated that contaminants (most notably nitrotriaminoanthraquinone) may be responsible since the chemical structure of Disperse Blue 1 is not typical of a genotoxic carcinogen. It was concluded, “Disperse Blue 1 cannot be considered to have intrinsic carcinogenic activity or potency”. It was further stated, “This conclusion supports the additional view that under conditions of actual use, this chemical does not present a carcinogenic risk to humans.”

FDA Risk Assessment Information – Presented in the Re-Review Document

In a 1994 memo, FDA staff presented an exposure assessment that incorporated dermal penetration data developed by Clairol. (The Clairol information was included in the original CIR report, and a brief summary is included in the ‘Dermal Absorption’ section of this re-review document.) In 1994, the FDA document indicated that skin absorption estimated by Clairol may have been underestimated, because there was a “lack of determination of the compound absorbed and remaining in the skin.” FDA staff therefore included a safety factor in their exposure assessment, resulting in a 10-fold increase in the exposure estimate. However, Yourick et al.7 confirmed that very little of the residual product in the skin penetrated the skin. Thus, skin content provides no significant contribution to the overall absorbed dose of disperse blue 1, and the 10-fold safety factor is not needed in the risk assessment.

The dermal penetration of disperse blue 1 measured by Yourick et al. was slightly greater than the Clairol value, 0.2% versus 0.15%. If 0.2% is used, the estimated lifetime absorbed-dose rate would be 0.44 µg/kg/day.

lifetime average daily exposure via hair dye use:

0.62 (g disperse blue 1 applied/application; assuming 100 ml formulation/application at a maximum concentration of 0.62% in the formulation) x 0.002 (fraction disperse blue 1 absorbed) x 600 (applications/lifetime) = 0.744 (g disperse blue 1 absorbed/lifetime)

0.744 g/60 kg = 0.0124 g/kg

78 yr (estimated lifespan) x 365 days = 28,470 days

estimated lifetime average daily absorbed-dose rate (hair-dye use): 0.0124 g/kg / 28,470 days = 0.44 µg/kg/day

A simple linear regression analysis was used to estimate that 0.3 µg/kg/day disperse blue 1 would be associated with an upper-bound 1 in 1,000,000 lifetime risk for developing a bladder cancer. The total lifetime daily exposure with hair dye use calculated above, 0.44 µg/kg/day, is slightly greater than that value. This risk assessment indicates that the human cancer risks associated with the use of disperse blue 1 in hair-coloring formulations would be negligible, considering multiple conservative assumptions incorporated into the assessment.

Additionally, the acceptable daily intake (ADI) reported in the FDA document, using the rat data from the NTP bioassay, was estimated to be 45 µg/kg/day, which is 100-times greater than the estimated lifetime daily dose.

As noted in the FDA 1994 memo, conclusions about the safe use of disperse blue 1 depend on whether its mechanism of action is viewed as genotoxic, thereby directly producing a carcinogenic event, or as a non-genotoxic. If it is viewed as non-genotoxic, the ADI and margin of safety indicated that potential exposure to disperse blue 1 would not increase the risk of developing cancer. If it is viewed as genotoxic, the estimated daily dose would be associated with an
upper-bound cancer risk estimate slightly exceeding $10^{-6}$ for humans, based on the approach described in FDA’s 1994 memo, as modified above (i.e., no uncertainty factor applied for insufficient dermal penetration data).

Industry-Prepared Quantitative Cancer Risk Assessment

A quantitative cancer risk assessment for disperse blue 1 was prepared based on the No Significant Risk Level (NSRL) of 200 µg/day established under Proposition 65 by the State of California. The NSRL was established using a linearized multistage model and the upper 95 percent confidence limit of the estimated cancer potency to extrapolate from the high oral doses used in the NTP rat bioassay to lower doses. Using the NSRL assumption of 70 kg as the adult human body weight, the equivalent average daily dose rate is 2.86 µg/kg/day. This risk assessment incorporated the dermal penetration estimate of 0.2%, as determined by Yourick et al. and described previously. For this risk assessment, the estimated lifetime average daily dose of disperse blue 1 from hair-dye use was determined as follows:

- amount of disperse blue 1 in contact with the skin = 41 µg/cm²
- amount systemically absorbed per use: 0.002 x 41 µg/cm² x 580 cm² (total scalp area) = 47 µg/application
- total lifetime exposure: 47 µg/application x 600 applications/lifetime x 1/60 kg bw = 470 µg/kg bw
- estimated lifetime average daily dose: 470 µg/kg bw/28,470 days (avg. female lifespan) = 0.0165 µg/kg/day

Thus, the estimated human exposure is ~170-fold lower than the NSRL (expressed as a lifetime average daily-dose rate) for disperse blue 1.

This risk assessment included a concentration of use value for disperse blue 1 of 0.52% in hair dyes. Since the existing CIR safety assessment of disperse blue 1 states that disperse blue 1 is safe at concentrations up to 1%, the risk assessment data were extrapolated to determine the margin of safety using 1% disperse blue 1. Assuming linear concentration-dependence, the lifetime average daily-dose rate from a 1% disperse blue 1 formulation would be 0.032 µg/kg/day, which is ~90-fold lower than the NSRL.

CIR has considered these inputs and offers this perspective on the risk assessments for genotoxic vs. non-genotoxic mechanisms of action:

**Assumptions: FDA Assessment, Disperse Blue 1 is Carcinogenic via a Genotoxic Mechanism**

- 0.62 g/100ml DB-1 in product
- 100ml/application
- 12 applications/year
- 50-year Exposure Duration
- 60-kg body weight
- 78 Years = 28,479 days Averaging Time for Non-Threshold Mechanisms

**Lifetime Average Daily-Dose Rate** (no correction for the fraction that actually contacts the scalp in a hair product)

\[
\frac{[0.62 \text{ g/100ml} \times 100\text{ml/application} \times 12 \text{ applications/year} \times 50 \text{ years}]}{[60 \text{ kg body weight} \times 28,479 \text{ days}]} = 2.18 \times 10^4 \text{ g/kg/day} \\
2.18 \times 10^4 \text{ g/kg/day} = 2.18 \times 10^2 \text{ µg/kg/day}
\]

- 0.002 (0.2%) of disperse blue 1 dermal penetration
  \[
  2.18 \times 10^2 \text{ µg/kg/day} \times 0.002 = 0.44 \text{ µg/kg/day}
  \]
• Uncertainty Factor = 10 for lack of data on the amount remaining in the skin, which could be absorbed;\(^24\) (Further investigation indicates that there is little potential for skin to serve as a reservoir,\(^7\) so this uncertainty factor is not needed.\(^25\))
\[
0.44 \text{ µg/kg/day} \times 10 = 4.4 \text{ µg/kg/day}
\]

• 0.02 (2\%) applied material contacts the scalp\(^24\)
\[
0.44 \text{ µg/kg/day} \times 0.02 = 0.009 \text{ µg/kg/day} \sim 0.01 \text{ µg/kg/day} (\text{without uncertainty factor})
\]
\[
4.4 \text{ µg/kg/day} \times 0.02 = 0.09 \text{ µg/kg/day} \sim 0.1 \text{ µg/kg/day} (\text{with uncertainty factor})
\]

Industry risk assessment estimate: 0.0165µg/kg/day (without uncertainty factor)\(^25\)

**Cancer Potency of Disperse Blue 1**

Extrapolated from an incidence of 41/49 (84\%) rats exhibiting smooth muscle cell tumors at 217 mg/kg/day exposure to estimate that 0.3 µg/kg/day is associated with a 10\(^6\) lifetime upper-bound cancer risk.\(^24\)

Note: A simple linear regression equation was used to extrapolate from the 217 mg/kg/day dose rate used in the NTP rat bioassay to low doses; the linearized multistage model or the upper 95\(^{th}\) percent confidence limit was not used to estimate cancer potency.

The State of California NSRL of 200 µg/day is based on: 25
- 70 kg body weight
- Linearized Multistage Model
- Upper 95\(^{th}\) percent confidence limit of the lowest dose that caused cancer in the NTP rat bioassay

NSRL = 200 µg/day / 70 kg = 2.86 µg/kg/day (represents <10\(^6\) lifetime cancer risk).

**Conclusion: Cancer Risk Assessment**

Exposure estimates above\(^24,25\) do not exceed lifetime average dose rates estimated to be associated with, at most, a 10\(^6\) lifetime cancer risk.

(MOS = 3 to 173)

**Assumption: Disperse Blue 1 is Carcinogenic via a Non-Genotoxic Mechanism**

Exposure averaging time for a non-genotoxic (i.e., threshold) effect is generally taken to be equal to the exposure duration. Assuming an averaging time (and exposure duration) of 50 years (rather than 78 years), the average daily dose rate would be (adjusting the estimates calculated above assuming a genotoxic mechanism):

\[
0.01 \text{ µg/kg/day} \times 78 \text{ years/50 years} = 0.016 \text{ µg/kg/day}
\]
\[
0.1 \text{ µg/kg/day} \times 78 \text{ years/50 years} = 0.16 \text{ µg/kg/day}
\]

Reference Dose Assuming a Non-Genotoxic Mechanism

NOAEL = 45 mg/kg/day (NTP rat bioassay)
Reference Dose = 45 mg/kg/day / 1,000 uncertainty factor = 45 µg/kg/day\(^24\)

(MOS = 281 to 2,812)

**Assumption: Concentration of Disperse Blue 1 is 1\% (Rather than 0.62\%\(^24\) or 0.52\%\(^25\))**

Exposure Estimates

\[
\sim 0.01 \text{ µg/kg/day} (\text{without uncertainty factor}), \text{assuming 0.62\%} \rightarrow
\]
\[
0.01 \text{ µg/kg/day} \times 1\%/0.62\% = 0.016 \text{ µg/kg/day at 1\%;} \sim 0.02 \text{ µg/kg/day} \sim 0.1 \text{ µg/kg/day (with uncertainty factor), assuming 0.62\%} \rightarrow
\]
0.1 µg/kg/day x 1%/0.62% = 0.16 µg/kg/day at 1%; ~ 0.2 µg/kg/day

0.0165 µg/kg/day (without uncertainty factor), assuming 0.52% ->
0.0165 µg/kg/day x 1%/0.62% = 0.0317; ~ 0.032 µg/kg/day

Thus, exposure estimates range from 0.02 to 0.2 µg/kg/day (the highest assuming an uncertainty factor of 10 for skin potentially serving as a “reservoir.”)

MOSs for Product Containing 1% Disperse Blue 1

Genotoxic Effect:
Range: [0.3 µg/kg/day / 0.2 µg/kg/day] to [2.86 µg/kg/day / 0.02 µg/kg/day] = 1.5 to 143

Non-Genotoxic Effect:
Range: [45 µg/kg/day / 0.2 µg/kg/day] to [45 µg/kg/day / 0.02 µg/kg/day] = 225 to 2,250

SUMMARY

Disperse blue 1 has previously been reviewed by the CIR Expert Panel, and, in 1995, the safety assessment was published with the conclusion that disperse blue 1 is “safe for use in hair dyes at concentrations up to 1%.” Evaluations of the carcinogenic potential of disperse blue 1 by the NTP and the IARC, which were not included in the 1995 assessment, have been summarized in this re-review. At the April 2010 meeting, the Expert Panel agreed to initiate a re-review of disperse blue 1 to re-evaluate its carcinogenic potential.

Disperse blue 1 is an anthraquinone color that functions in cosmetics as a direct (semi-permanent) hair colorant. In 1994, it was reported to the FDA through the VCRP that disperse blue 1 was used in 112 hair color formulations at most at a concentration of 0.62%. There are now zero uses in the VCRP, and industry has reported no use of disperse blue 1.

The 1995 safety assessment concluded that disperse blue 1 was poorly absorbed through the skin. The dermal penetration of formulations containing 1% disperse blue 1, tested in vitro using skin from miniature pigs, did not exceed 0.15% of the dose. In the current safety assessment, it was found that 0.2% of the dose of disperse blue 1 applied to human skin in vitro was found in the receptor fluid. This was the same with both an ethanol and a spiked semi-permanent vehicle. However, the vehicle did affect the amount of dose that remained in the skin. Using rat skin, 0.4-0.7% of the dose was found in the receptor fluid. Little of the disperse blue 1 that penetrated the skin within 24 h moved into the receptor fluid over 72 h.

The oral LD₅₀ of disperse blue 1 in corn oil was >3000 mg/kg for rats and >2000 mg/kg for mice. In a study in which rats were dosed with 1 g/kg disperse blue 1 by gavage for 1-3 days or with 1% in feed for 4 days, dye was found in the kidneys of rats dosed by gavage, and hyperplasia of the renal pelvis epithelium and nephropathy were observed. For the rats fed disperse blue 1 in feed, there was no accumulation in the kidneys, but there was low-grade hyperplasia of the bladder urothelium. In a 14-day dietary study with ≤50,000 ppm disperse blue 1, some mid-dose groups of rats and mice had decreased body weight gains, the high-dose rats and mid-dose mice were inactive, and all test animals excreted blue urine and had blue organs at necropsy. In a 13-wk dietary study in which rats were fed ≤20,000 ppm and mice were fed ≤10,000 ppm disperse blue 1, the higher dose group rats and mice had decreased final body weights, all test animals excreted blue urine. In this study, male and female rats and mice fed diets containing ≥2500 ppm had pigmentation of the thyroid gland follicle, renal pigmentation, lesions in the urinary bladder, and urinary tract calculi, and mice had nephrosis and focal myocardial necrosis. In a 2-yr study, no adverse effects were observed when dogs were fed 19.5 or 97.5 mg/kg of a formulation containing 1.54% disperse blue 1.
The irritation threshold for disperse blue 1 was > 10%, and 1% disperse blue 1 was a moderate sensitizer in studies with guinea pigs. Disperse blue 1, at 3%, was a moderate sensitizer in a LLNA using mice.

No adverse reproductive or teratogenic effects were observed in feed or gavage studies using rats and/or rabbits of dye formulations containing 1.54% disperse blue 1.

In Ames tests, disperse blue 1 was mutagenic to some strains of Salmonella typhimurium, but conflicting results were sometimes obtained between studies. Positive results were also obtained in an L5178Y mouse lymphoma cell mutation assay (concentrations of ≤160 µg/ml), a chromosome aberration test using Chinese hamster ovary (CHO) cells (at concentrations of ≥7.5 µg/ml with metabolic activation and ≥9 µg/kg without), and a sister chromatid exchange test (at concentrations ≥3.3 µg/ml without metabolic activation). A semi-permanent hair dye containing 0.12% disperse blue 1 was evaluated in an in vivo heritable translocation study, and no effects were seen.

In a carcinogenicity bioassay in which rats and mice were fed diets containing ≤5000 ppm and ≤2500 ppm disperse blue 1, respectively, disperse blue 1 was carcinogenic in male and female rats. It increased the incidence of transitional cell papillomas and carcinomas, of leiomyomas and leiomyosarcomas, and of squamous cell papillomas and carcinomas of the urinary bladder. Equivocal results were obtained in tests with male mice; neoplastic lesions included hepatocellular adenomas or carcinomas (combined) in low and mid-dose males and alveolar/bronchiolar adenomas or carcinomas (combined) in high dose males. Negative results were reported in female mice. In another dietary study, rats were given diets containing ≤0.1% disperse blue 1 for 19 mos or 1.0% for 6 mos. The rats fed 1.0% developed lesions of the urinary bladder and kidneys, which were associated with dye deposits and calculi. However, no sarcomas of the bladder wall or epithelial neoplasms were observed. Results of a dermal carcinogenicity study, evaluating two non-oxidative hair dye formulations containing 0.1 or 0.3% disperse blue 1, were negative. Three compounds that are structurally-related to disperse blue 1, i.e. 2-aminoanthraquinone, 1-amino-2-methylanthraquinone, and 2-methyl-1-nitroanthraquinone, were carcinogenic to animals. Two of the chemicals affected the liver, while the third affected the stomach and bladder.

In the 1995 assessment, it was reported that 1 of 15 patients with contact dermatitis from textile dyes had a positive patch-test reactions to disperse blue 1. In the current safety assessment, one case study in which reactions to black “velvet” clothes were reported, strong reactions to disperse blue 1 were not observed.

The NTP found disperse blue 1 is reasonably anticipated to be a human carcinogen, and the IARC found that there was sufficient evidence of carcinogenicity in animals to conclude that disperse blue 1 is possibly carcinogenic to humans.

The 1995 CIR safety assessment stated that risk assessments using both biologic and quantitative approaches indicate that hair dye use of disperse blue 1 does not pose a carcinogenic risk to humans. Safe exposure concentrations determined in the NTP bioassay were significantly greater than the estimated maximum lifetime average daily applied dose associated with the use of semi-permanent hair dyes. Both risk assessments indicated the belief that disperse blue 1 was not acting through a direct genotoxic mechanism, and that any weak mutagenic activity that occurred could be due to mutagenic contaminants. In a risk assessment performed by the FDA, the estimated lifetime hair dye use daily dose of disperse blue 1 is 0.44 µg/kg/day. This value is slightly greater than the value estimated to be associated with a 1:1,000,000 lifetime upper-bound cancer risk, which was 0.3 µg/kg/day. The acceptable daily intake, based on the results of rat bioassay conducted by the NTP, and the assumption of a non-genotoxic mechanism, is 100 times greater than the estimated lifetime average daily-dose rate. Risk estimates for the use of disperse blue 1 depend on whether it is viewed as a genotoxic or non-genotoxic agent. In a third risk assessment prepared using a the NSRL of 200 µg/day established under Proposition 65 in the State of California, the estimated human exposure from a hair dye containing 1% disperse blue 1 is approximately 90-fold lower than the NSRL.
The most recent comprehensive review of available epidemiology studies concluded that there is insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers. (A summary of the available hair dye epidemiology data is available at [http://www.cir-safety.org/findings.shtml](http://www.cir-safety.org/findings.shtml)) However, the NTP stated in its 11th Report on Carcinogens that disperse blue 1 is “reasonably anticipated to be a human carcinogen based on evidence of malignant tumor formation in experimental animals to an unusual degree with regard to incidence, site, and type of tumor (NTP 1986)\(^2\), and because it is an anthraquinone and, therefore, structurally related to other substances listed in a previous Annual Report on Carcinogens as either known to be human carcinogens or reasonably anticipated to be human carcinogens (NTP 1994).” The IARC concluded that there was sufficient evidence for the carcinogenicity of disperse blue 1 in experimental animals for an overall evaluation that “Disperse Blue 1 is possibly carcinogenic to humans (Group 2B).”

**DISCUSSION**

The Panel was concerned that the current conclusion for Disperse Blue 1 reflected its use at 1% concentration of use rather than a 0.3% concentration of use. Further, since the mechanism of smooth muscle cell tumors was not known, the Panel wanted a Risk Assessment based on a potential genotoxic mechanism of action (rather than a threshold effect) and asked that the risk assessment and margin of safety be redone.

The report presented risk assessments based on a range of assumption and based on genotoxic and non-genotoxic mechanisms of action. Margins of Safety (MOS) were calculated from both assessments. Based on an assumption of a genotoxic effect, the MOSs for Product Containing 1% Disperse Blue 1 would range between 1.5 to 143. Based on a non-genotoxic effect, the MOS range would be 225-2250.

Remainder to be developed at the Expert Panel meeting.

**CONCLUSION**

To be determined at the Expert Panel meeting.
Figure 1. Disperse Blue 1

![Disperse Blue 1](image)

Table 1. Synonyms of disperse blue 1

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Synonyms/Other Technical Names</th>
</tr>
</thead>
<tbody>
<tr>
<td>disperse blue 1</td>
<td>9,10-anthracenedione, 1,4,5,8-tetraamino-&lt;sup&gt;6,26&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>anthraquinone, 1,4,5,8-tetraamino-&lt;sup&gt;(8CI)&lt;/sup&gt;&lt;sup&gt;26&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>CI 64500&lt;sup&gt;6,26&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Solvent Blue 18&lt;sup&gt;6,26&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1,4,5,8-tetraamino-9,10-anthracenedione&lt;sup&gt;6,26&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1,4,5,8-tetraaminoanthraquinone&lt;sup&gt;26&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1,4,5,8-tetraminoanthraquinone&lt;sup&gt;26&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Table 2. Historical and current use of disperse blue 1

<table>
<thead>
<tr>
<th>Product Category</th>
<th>Freq. of use – 1994 (# in category)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Freq. of use – 2010 (personal communication, D. Havery)</th>
<th>Conc. of use 1994&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Conc. of use 2009&lt;sup&gt;5&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>hair dyes and colors</td>
<td>110 (1458)</td>
<td>0</td>
<td>0.62%*</td>
<td>none reported</td>
</tr>
<tr>
<td>other hair coloring prep.</td>
<td>2 (73)</td>
<td>-</td>
<td>-</td>
<td>none reported</td>
</tr>
<tr>
<td>Totals</td>
<td>112</td>
<td>3</td>
<td>0.62%*</td>
<td>none reported</td>
</tr>
</tbody>
</table>

*in semi-permanent hair dyes as reported by industry
REFERENCES


12. National Toxicology Program. Toxicology and carcinogenesis study of C.I. Disperse Blue 1 (a commercial hair dye containing approximately 50% 1,4,5,8-tetraaminoanthraquinone, 30% other compounds structurally related to 1,4,5,8-tetraaminoanthraquinone, a nd 20% water) in F344/N rats and B6C3F1 mice feeding studies. (Technical report No. 299.) Research Triangle Park, NC: U.S. Department of Health and Human Services. 1986.


http://chem.sis.nlm.nih.gov/chemidplus/ProxyServlet?objectHandle=Search&actionHandle=getAll3DMViewFiles&nextPage=jsp%2Fcommon%2FChemFull.jsp%3FcalledFrom%3Dlite&chemid=002475458&formatType=_3D Website accessed Nov. 13, 2009.:
Memorandum

TO: F. Alan Andersen, Ph.D.
    Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Personal Care Product Council Hair Colorants Technical Committee

DATE: November 8, 2010

SUBJECT: Quantitative Cancer Risk Assessment on Disperse Blue 1
Summary

A quantitative cancer risk assessment has been conducted for Disperse Blue 1 in response to a request from the CIR Expert Panel. The No Significant Risk Level of 200 μg/day published by the State of California under Proposition 65 was used for this risk assessment. Lifetime daily human exposure associated with use of a semi-permanent hair dye containing Disperse Blue 1 was estimated from a published in vitro dermal penetration study in human skin and conservative assumptions on hair dye usage habits and practices. The quantitative risk assessment indicates that estimated human exposure is approximately two orders of magnitude lower than the No Significant Risk Level for carcinogenicity. An evaluation of areas of uncertainty in the exposure assessment indicates that even with the use of extreme assumptions, which are likely to significantly overestimate exposure, the estimated human exposure is comparable to or lower than the No Significant Risk Level. This assessment supports the conclusion that the use of Disperse Blue 1 in semi-permanent hair dyes does not pose a significant cancer risk.

Background

In 1995 the CIR Expert Panel published a safety assessment on Disperse Blue 1 and concluded that the ingredient was safe for use in hair dyes at concentrations up to 1% (1). This safety assessment evaluated the results from the NTP rodent carcinogenicity studies (2) and subsequent studies conducted by Burnett and Squire (3). These studies demonstrate that Disperse Blue 1 induces urinary bladder tumors (transitional cell and squamous cell papillomas as well as leiomyomas and leiomyosarcomas) in male and female rats. The CIR safety assessment also reviewed the quantitative cancer risk assessment provided by industry, which was also published in the peer reviewed literature (4). This risk assessment derived safe exposure estimates based on either a threshold approach, which assumed that the mode of action of urinary bladder tumors in rats was related to the development of urinary calculi, or on a conventional quantitative risk assessment approach which assumed linear low dose extrapolation. The industry estimate of human exposure assumed that semi-permanent hair dyes contained a maximum concentration of 0.62% Disperse Blue 1 (4). The CIR safety assessment concluded that Disperse Blue 1 does not pose a carcinogenic risk to humans because safe exposure concentrations were significantly greater than the estimated maximum lifetime exposure associated with the use of semi-permanent hair dyes (1).

Subsequent to the publication of the CIR safety assessment, in 1996 the NTP report on Carcinogens (Eleventh Edition) evaluated the carcinogenicity of Disperse Blue 1 (5). The report reviewed evidence from experimental animal carcinogenicity studies and stated that the animal data “suggest that the transitional cell and squamous cell tumors
induced by Disperse Blue 1 in the urinary bladder would not occur in humans exposed to amounts of Disperse Blue 1 insufficient to also cause bladder calculi.” However, the report also stated that “compelling data that demonstrate a causal relationship between urinary bladder calculi and leiomyomas and leiomyosarcomas have not been sufficiently developed to contradict other evidence that Disperse Blue 1 is reasonably anticipated to be a human carcinogen.”(5).

Disperse Blue 1 has recently come up for re-review by the CIR Expert Panel. This ingredient is not currently used in hair dye formulations marketed in the U.S. Nevertheless, an expert report concerning the carcinogenicity of Disperse Blue 1 was prepared by Samuel M. Cohen, M.D., Ph.D. at the request of industry, and this report was provided to the CIR Expert Panel prior to their August 30-31, 2010 meeting. Dr. Cohen concluded that Disperse Blue 1 unquestionably produces bladder tumors in rats and that considerable information is available to suggest that this is associated with the formation of calculi which leads to cytotoxicity, regenerative proliferation, and ultimately tumor formation. However, Dr. Cohen also concluded that there are some remaining questions concerning the mode of action for induction of smooth muscle tumors (leiomyomas and leiomyosarcomas). Such tumors have not been observed in association with urinary calculi for other compounds that produce transitional cell and squamous cell tumors in the urinary bladder in rats as a result of urinary calculi formation. In addition, since Disperse Blue 1 gives weakly positive responses in some in vitro genotoxicity assays (which may be due to impurities) and the in vivo genotoxicity potential of Disperse Blue 1 has not been adequately evaluated, it remains unclear whether the carcinogenic effects of Disperse Blue 1 in the rat might be related, at least partially, to genotoxicity.

At the August 30-31, 2010 CIR Expert Panel meeting, the report on Disperse Blue 1 was tabled and the Expert Panel requested a calculation of a margin of safety for carcinogenicity for this ingredient (6). The following provides a quantitative cancer risk assessment for carcinogenicity for Disperse Blue 1.

**Carcinogenic Potency Assessment**

Disperse Blue 1 is included in the list of compounds known to the State of California to cause cancer (Proposition 65), and a No Significant Risk Level (NSRL) of 200 µg/day has been published (7). This NSRL assumes an adult human body weight of 70 kg; therefore the NSRL is equivalent to an exposure of 2.86 µg/kg/day. In its Proposition 65 carcinogenicity risk assessments, the State of California assumes the absence of a carcinogenic threshold dose and uses a linearized multistage model for extrapolation from high to low doses, taking the upper 95th percent confidence limit for potency determination (8). This represents a conservative approach for assessment of Disperse Blue 1, since this compound may be acting via a threshold mode of action (urinary calculi formation). Nevertheless, linear low dose extrapolation will be assumed, given the uncertainties regarding the mode of action of Disperse Blue 1 discussed above. Furthermore, although Disperse Blue 1 is not currently used in products marketed in the US, any future use in California would require compliance with Proposition 65, and the NSRL value published by the State of California would be used to assess compliance.
Therefore, for the quantitative cancer risk assessment presented here the value of 200 μg/day (2.86 μg/kg/day) will be used as an exposure that does not present a significant cancer risk.

**Exposure Assessment**

**Dermal Penetration**

Yourick et al. conducted an in vitro dermal penetration study with [14C]-Disperse Blue 1 in viable split thickness (200-280 μm) human skin using flow-through diffusion cells (9). In this study, a semi-permanent hair dye formulation containing 0.52% Disperse Blue 1 was applied to the skin at a dose of 7.8 mg formulation/cm² skin. After 30 minutes (an exposure period relevant for hair dye use), the skin was washed to remove the dosing formulation and unabsorbed material. Receptor fluid was collected at 3-6 hour intervals up to 24 hours. After 24 hours, the skin was removed from the diffusion cells and skin discs were tape stripped to remove the stratum corneum. Tape strips, the remaining viable epidermis/dermis and receptor fluid samples were analyzed by liquid scintillation counting.

Disperse Blue 1 was poorly absorbed, and the amount found in receptor fluid represented 0.2% of the applied dose. Approximately 3% of the applied dose remained in the skin, but approximately 80% of this was reported to be in the stratum corneum. With further investigation, it was determined that there was little potential for the skin to serve as a reservoir for further absorption of Disperse Blue 1. Therefore, the authors concluded that it was not appropriate to add the skin levels of Disperse Blue 1 to the receptor fluid levels to determine the total systemically absorbed amount. Therefore, for exposure estimates the percent of applied dose considered to be absorbed was 0.2% (9).

The results of Yourick et al. (2004) are similar to the results reported in a previous in vitro dermal penetration study conducted in miniature pig skin where 0.15% of the applied amount was recovered in the receptor fluid (10). The amount remaining in the skin was not measured in this study.

The results of Yourick et al. (2004) are used for the estimation of Disperse Blue 1 systemic exposure following use of semi-permanent hair dye since this study is the more robustly designed and thoroughly reported study.

**Exposure Assumptions**

- 12 uses of semi-permanent hair dye per year for 50 years. 12 X 50 = 600 lifetime uses (Assumptions applied by FDA) (11). This is more conservative than the assumptions in the previous industry assessment (10 uses/year X 40 years) (4)

- Average female lifespan = 78 years (28,470 days). This is an updated number from the default value of 70 years and is based on the EPA Exposure Factors Handbook,
July 2009 Update (12)

- Average female body weight = 60 kg

- In vitro conditions used by Yourick et al (2004) (9) are representative of human exposure conditions for hair dye use (30 minute exposure to a semi-permanent hair dye formulation; skin contact is 7.8 mg formulation/cm² skin)

- 0.52% Disperse Blue 1 is used in semi-permanent hair dye (concentration used in the Yourick et al (2004) dermal penetration study) (9)

- Total scalp surface area exposed during hair dye use = 580 cm² (13)

Calculations (assuming 0.2% systemic absorption)

Amount of DB1 in contact with skin per use (from Yourick et al., 2004) (9):

\[
\frac{0.52 \text{ g DB1}}{100 \text{ g formulation}} \times 10^6 \text{ ug} \times 1 \text{ g} \times 7.8 \text{ mg formulation/cm}^2 \text{ skin} = 41 \mu\text{g/cm}^2
\]

Amount of DB1 systemically absorbed per use:

0.002 \times 41 \mu\text{g/cm}^2 \times 580 \text{ cm}^2 = 47 \mu\text{g per use}

Total lifetime exposure:

47 \mu\text{g/use} \times 600 \text{ uses} \times 1/60 \text{ kg body weight} = 470 \mu\text{g/kg body weight}

Estimated lifetime daily dose:

470 \mu\text{g/kg body weight} / 28,470 \text{ days} = 0.0165 \mu\text{g/kg/day}

Risk Assessment

Comparison of NSRL to Estimated Human Exposure

NSRL / Estimated Human Exposure = 2.86 \mu\text{g/kg/day} / 0.0165 \mu\text{g/kg/day} = 173

Based on these calculations, estimated human exposure is \sim 170 fold lower than the No Significant Risk Level for carcinogenicity determined by the State of California for Disperse Blue 1.
Maximum Concentration of Use

The original CIR safety assessment concluded that Disperse Blue 1 is safe for use in hair dyes at concentrations up to 1% (1). The present quantitative cancer risk assessment was conducted assuming a concentration of 0.52% (the concentration used in the in vitro dermal penetration study). In that study, the amount of Disperse Blue 1 absorbed through the skin was presented as a percentage of the applied dose (9). Since percent of applied dose can be a misleading representation of the data, especially when extrapolating from one applied dose to another, for the present exposure assessment the data from Yourick et al. (2004) (9) were used to express the absorbed dose in units of μg/cm² and then the amount absorbed per hair dye use (47 μg) was calculated by multiplying by the total scalp surface area.

Extrapolation from a concentration of Disperse Blue 1 in hair dye of 0.52% to 1% would require a further assumption on the concentration dependence of dermal penetration. If one assumes linear concentration dependence within the range of 0.52% and 1%, then the amount of Disperse Blue 1 systemically absorbed per use would be 90 μg, and the lifetime daily dose, assuming 600 uses, would be 0.032 μg/kg/day. This estimated human exposure is ~90 fold lower than the NSRL. Given the large margin of safety, the uncertainty in extrapolating between the two concentrations is of minimal concern.

Areas of Uncertainty in Exposure Assessment

In this assessment the dermal penetration results from an in vitro study in human skin published by Yourick et al. (2004), who reported that 0.2% of the applied dose was absorbed, were used in the exposure assessment (9). Haws et al. (1994) assumed that 2% of the total amount of Disperse Blue 1 in 100 g of formulation applied to the hair contacts the scalp and that 100% of the amount in contact with skin is absorbed (4). In the memorandum from Yourick and Bronaugh (1994), 1.5% of the total Disperse Blue 1 applied to the head was assumed to be absorbed (11). The average lifetime daily exposures estimated by Haws et al. (1994) and Yourick and Bronaugh (1994) were 2.7 and 3.3 μg/kg/day, respectively, assuming the concentration of Disperse Blue 1 in the formulation was 0.62%. These dermal absorption assumptions were made in the absence of a well-conducted in vitro skin penetration study and are extremely conservative in light of the newer skin penetration data. Nevertheless, these exposure estimates are comparable to the NSRL of 2.86 μg/kg/day.

Yourick et al. (2004) concluded from their in vitro skin penetration studies that the amount of Disperse Blue 1 found in the skin (stratum corneum, epidermis, and dermis) does not serve as a reservoir for further absorption and that the amount found in the receptor fluid (0.2% of the applied dose) should be considered as the systemically absorbed dose (9). While this conclusion is supportable based on the data presented, even if a more conservative approach is used by taking the amount found in the receptor fluid plus viable skin (epidermis and dermis) (0.2 % + 0.62% = 0.82%) or in the receptor
fluid plus total skin (stratum corneum, epidermis, and dermis) (0.2% + 3.1% = 3.3%) as the potentially absorbed dose, the estimated lifetime daily doses would be 0.068 and 0.27 μg/kg/day, respectively. These exposure estimates are also below the NSRL.

The present assessment assumes 12 uses of semi-permanent hair dye per year for a duration of 50 years, resulting in a total of 600 uses over a 78 year lifespan. These are the same assumptions used in the 1994 memorandum by Yourick and Bronaugh (11) who cited IARC (1993) (14). Regarding duration of use, 50 years is considered a very conservative assumption. Regarding frequency of use, semi-permanent hair dyes last through 6-12 shampoos, and IARC (1993) stated that users either apply such products at least monthly or use them only on special occasions, for example, three to four times per year (14). If one very conservatively assumes a frequency of use of once per week rather than once per month, the estimated lifetime daily dose of Disperse Blue 1 would be increased by a factor of 4.3 fold but would still be below the NSRL.
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11. March 8, 1994 DHHS memo from JJ Yourick and RL Bronaugh re. Review of Disperse Blue 1 Data (DSAT Assignment No. 006)


Memorandum

TO: F. Alan Andersen, Ph.D.
   Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: John Bailey, Ph.D.
       Industry Liaison to the CIR Expert Panel

DATE: August 11, 2010

SUBJECT: Expert Report Concerning Carcinogenicity of Disperse Blue 1

August 11, 2010

Expert Report Concerning Carcinogenicity of Disperse Blue 1

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I. INTRODUCTION

Disperse Blue 1 is an anthraquinone dye that has been used as a hair colorant in the past. A 2-year bioassay in rats and mice performed by the National Toxicology Program (NTP) (NTP, 1986) demonstrated tumors of the urinary bladder in rats, both male and female, but without tumorigenic effects in mice. Similarly, a more focused study evaluating carcinogenicity by Burnett and Squire (1986) also demonstrated bladder carcinogenicity in male and female rats, with detailed information regarding effects on calculus formation, kidney and bladder proliferation, and possible reversibility. Numerous genotoxicity studies have been performed with variable results.

Unquestionably, the agent produces bladder tumors in rats. Considerable information is available to suggest that this is associated with the formation of calculi which produces cytotoxicity, regenerative proliferation, and tumors (NTP, 1986; Burnett and Squire, 1986; Haws et al., 1994; Cosmetic Ingredient Review, 1995). However, there are some remaining questions which need to be addressed to convincingly demonstrate this as the mode of action for all of the tumor types, and more importantly, to exclude other possible modes of action, such as genotoxicity. These will be discussed in detail.

II. CHEMICAL FORMULATION

As produced commercially Disperse Blue 1 is actually a mixture of chemicals. The principle chemical is 1,4,5,8-tetraaminoanthraquinone (TAQ). However, chemical analysis of the commercial product demonstrated that there were several impurities, of which approximately 30% are structurally related compounds and approximately 20% is water. Commercial preparation contains approximately equal amounts of dye stuff and lignosulfonate dispersants (Burnett and Squire, 1986; NTP, 1986). Amounts of the specific dye stuff and dispersants vary by commercial products.

One of the major contaminants (approximately 6% of the dye) has been identified as triaminonitroanthraquinone (TANAQ) (NTP, 1986).
III. NATIONAL TOXICOLOGY PROGRAM 2-YEAR BIOASSAY

Disperse Blue 1 (minus lignosulfonate dispersants) was tested for carcinogenicity by the NTP (1986). Groups of 50 male and 50 female F344 rats and B6CF1 mice were administered the chemical in the diet for up to 2 years. The dose that was used for the rats was 1250, 2500 or 5000 ppm Disperse Blue 1, and in the mice the doses were 600, 1200 or 2500 ppm Disperse Blue 1. Control groups were administered the same diet but without chemical treatment.

In the mice, there was evidence of inflammation and epithelial hyperplasia in the urinary bladder, calculi in the urinary bladder lumen, and fibrosis of the urinary bladder wall at high doses (NTP, 1986). There was also cast material in the renal tubular lumina as well as renal tubular degeneration, although it was unclear whether this could be related to chronic aging nephrothropy that can occur in mice, similar as in rats. However, there was no evidence of an increased incidence of bladder tumors or tumors at any other site in the mice. Thus, the carcinogenicity assay in mice was negative.

Similar non-neoplastic changes were seen in the kidney and urinary bladder of the male and female rat, including significant toxicity, ulceration of the bladder epithelium, as well as the presence of urinary calculi in the lumen (NTP, 1986; Burnett and Squire, 1986). However, in contrast to the mice, a significant number of male and female rats also developed urinary bladder neoplasms. Of significance, these neoplasms included not only the usual urothelial tumors associated with calculi, such as transitional cell and squamous cell papillomas and carcinomas (Oyasa, 1995), but also included a very high incidence of leiomyomas and leiomyosarcomas (NTP, 1986). There was also evidence of calculus material in the epithelium and submucosa of the bladder, as well as extensive chronic inflammation.

The incidence of epithelial tumors was increased at the doses of 2500 and 5000 ppm, and an increase was also identified of the smooth muscle tumors at these same doses. Of interest, the smooth muscle tumors occurred at a significantly higher incidence than the epithelial tumors. Incidences of transitional cell papilloma and carcinoma, squamous cell papilloma or carcinoma, and smooth muscle tumors are listed in Table 1.

Table 1: Urinary bladder tumors in rats administered Disperse Blue 1 for 2 years (National Toxicology Program, 1986).

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Incidences(^a) at Listed Dose(^b)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Males</td>
</tr>
<tr>
<td>0 ppm</td>
<td>0/49</td>
</tr>
<tr>
<td>1250 ppm</td>
<td>0/50</td>
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<tr>
<td>2500 ppm</td>
<td>8/50</td>
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<td>5000 ppm</td>
<td>4/49</td>
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<tr>
<td>0 ppm</td>
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<td>1250 ppm</td>
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<td>2500 ppm</td>
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<tr>
<td>1250 ppm</td>
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<tr>
<td>2500 ppm</td>
<td>7/50</td>
</tr>
<tr>
<td>5000 ppm</td>
<td>41/49</td>
</tr>
</tbody>
</table>

\(^a\) Incidences are listed as number of animals with tumor type/total number of animals.
\(^b\) Dose is in ppm of the diet.
Several years ago I had the opportunity to discuss and review these smooth muscle tumors with Dr. Robert Maronpot who was the head of pathology at the National Toxicology Program bioassay program. He requested this review because of the unusual nature of the tumor type being produced in this study. Unquestionably, these tumors are leiomyomas and leiomyosarcomas, and are not epithelial tumors.

It was noted in the NTP report that most of the animals with tumors had calculi, although not all. The NTP as well as others evaluating studies involving calculi have commented that it is not unusual for calculi to be lost during the course of an experiment, even after tumors have been produced (NTP, 1986; Clayson et al., 1995; Cohen et al., 2007). Thus, although there is not a 100% correlation between the presence of calculi in tumors, it is accepted that the relationship is more than can be established simply by the presence or absence of calculi at the time of terminal sacrifice. This was best demonstrated in a study with Fosetyl-A1 (Phang and Rinde, 1993). At the terminal sacrifice, there was a marked increase in bladder tumors, and some of the animals with tumors had associated calculi, but many did not. However, in a subsequent study examining the urinary bladders of animals administered Fosetyl-A1 for shorter periods of time (weeks rather than years), there was nearly a 100% incidence of bladder calculi present.

IV. BURNETT AND SQUIRE BIOASSAY

Burnett and Squire (1986) performed an investigation examining not only the carcinogenicity of Disperse Blue 1 administered in the diets to rats, but also to examine short term effects on the production of calculi, urinary bladder toxicity and proliferation, as well as reversibility. They examined animals that were administered Disperse Blue 1 at doses of .01 and .1% for 19 months and 1% for 6 months. They also examined several doses by gavage for periods of up to 4 days and administration as 1% of the diet for 4 days to evaluate short term effects.

They, like the NTP, observed bladder tumors at the highest doses, and found that these were associated with the presence of calculi. In the short term studies, the rats administered the agent by gavage were observed to have dye present in the kidneys at days 2-4, with associated toxicity and proliferation. In contrast, the urinary bladder of the gavage-administered animals did not show abnormalities. However, the rats administered Disperse Blue 1 as 1% in the diet demonstrated toxicity and hyperplasia in the bladder by day 4.

There were several other aspects that were evaluated in this study that are important for the overall risk assessment. To begin with, they state, but did not provide the data, that the same effect was seen with administration of the dye with or without the dispersant. Clearly, it is the dye that is producing the urinary bladder effects.

Furthermore, they evaluated the potential reversibility of the changes produced by Disperse Blue 1 in the urinary bladder. The calculi were surgically removed, and then the animals observed for several weeks before being sacrificed for examination of their urinary bladders. Somewhat surprisingly, the bladder incidence of tumors increased in both the males and females by about two-fold in the animals that had the calculi surgically removed in contrast to animals that did not have the calculi surgically removed. Furthermore, in the males, the dye-containing calculi were
nearly absent following surgery and the bladder toxicity and proliferation appeared to attenuate, with the epithelium returning to normal. In contrast, the number of calculi in the females actually increased following surgical removal, with associated increased toxicity and proliferation.

Critical to the evaluation of this experiment was their identification that the calculi in the males were composed nearly entirely of the dye whereas in females the major component was calcium phosphate, with only small amounts of the dye present. With surgical removal, there is suture material present in the wall of the bladder. This is well known to be associated with an increased potential for formation for calcium-containing urinary solids, including calculi (Clayson et al., 1995). This appears to be what happened in the females in this reversibility study. It does imply, however, that there may have been an irreversible change induced by the administration of the chemical on the homeostatic balance in the urine regarding calcium solubility.

For all aspects of this study, they emphasize that there was a close correlation between the presence of calculi and eventual development of the epithelial tumors. However, they only observed epithelial tumors in this particular study in contrast to the NTP study. In some animals, however, they did observe crystals or calculi imbedded in the wall of the bladder, even through the full thickness of the muscle wall and into the adjacent subserosal tissue, and these solids frequently were associated with an increase in a histiocytic infiltration around the foreign material. However, they did not see any actual leiomyomas or leiomyosarcomas. They attribute this difference between their study and the NTP as differences in the dose and especially significant differences in the length of the experiment. The longest period of examination in their experiment was 19 months at the two lower doses, and only 6 months for the highest dose. This major difference in time between their study and the NTP study likely plays a significant role in the lack of smooth muscle tumors in their study. However, in the NTP study, there were occasional smooth muscle tumors observed prior to the terminal sacrifice.

V. GENOTOXICITY ASSAYS

The genotoxicity of Disperse Blue 1 has been evaluated in a variety of in vitro assays. In the Ames test, it was positive in strains TA98 and TA97 with and without metabolic activation, whereas it was positive in strain TA1535 only in the presence of S9. It was not mutagenic in strain TA100 (Zeiger et al., 1988). In another study using the Ames assay, it showed equivocal results with strain TA1537 with and without metabolic activation, but was negative in strains TA1538, TA1535, TA100, and TA98 (Brown and Brown, 1976). Disperse Blue 1 was also positive in the L5178Y mouse lymphoma cell mutation assay (Myhr et al., 1990) and was positive in the chromosome aberration test utilizing Chinese hamster ovary (CHO) cells (Anderson et al., 1990). Sister chromatid exchange evaluation was also positive in CHO cells without metabolic activation, but was only weakly to marginally positive with metabolic activation (Anderson et al., 1990).

The results of these genotoxicity studies with Disperse Blue 1 are complicated by the fact that the agent actually is a mixture and, most importantly, contains as a major contaminant a nitroantraquinone. Nitroaromatic chemicals are particularly important in this overall consideration, since they are frequently strongly positive in the standard genotoxicity assays
(Purohit and Basu, 2000), primarily because of the spontaneous reduction of the nitro group by
the plentiful and active bacterial or cellular nitroreductase. This likely is the explanation for the
positive results with and without S9, the results being weaker in the absence of S9 activation.
This has been an issue with other anthraquinones, also. Thus, it remains unclear as to which of
the anthraquinones are truly genotoxic, particularly those that do not have the nitro group
present.

There is evidence, however, that the amino groups in some of the anthraquinones, such as TAQ,
the major ingredient in Disperse Blue 1, can be metabolically activated by ring hydroxylation or
N-hydroxylation (Doi et al., 2005; Gothoskar et al., 1979). Although glucuronidation rapidly
occurs, there is the potential for the N-hydroxylamine to be present in the urine and to react with
the urinary bladder, similar to aromatic amines. I am not aware, however, of any assessment of
the urothelium for the presence of DNA adducts. Similar to aromatic amines, not all
anthraquinones appear to be metabolically N-hydroxylated.

VI. SKIN CARCINOGENESIS BIOASSAY AND ABSORPTION

A carcinogenicity assay has been performed by topical administration to mice, and this was
shown to be negative. However, this is not surprising given the negative findings in the 2-year
bioassay in mice involving oral administration. The mouse skin carcinogenicity study (Jacobs et
al., 1984) involved administration of the agent to the interscapular area of the back of mice (60
males and 60 females) 3 times per week for 20 months. There were no differences in non-
neoplastic and neoplastic responses between the treated and control groups of mice. However,
unfortunately, there was not a detailed description of the findings in the kidney or bladder in this
study. Given the level of exposure, poor dermal absorption and comparison of this dose with that
used in the NTP oral administration study, one would not anticipate there to be an effect on the
urinary tract of these mice.

Dermal absorption studies have also been performed, demonstrating that there is very little
absorption across the skin. This was evaluated in in vitro model systems utilizing human and rat
skin. Disperse Blue 1 was evaluated utilizing ethanol as a vehicle as well as the commercial
formulation as a vehicle (Yourick et al., 2004).

VII. OTHER ANTHRAQUINONES

The NTP has undertaken an evaluation of several anthraquinones to determine some possible
structural activity relationships regarding carcinogenicity (Doi et al., 2005). Seven chemicals
have been evaluated in the standard two year bioassays, including Disperse Blue 1. The results of
the study with Disperse Blue 1 are described above. The other chemicals that have been
evaluated include anthraquinone itself, 2-aminoanthraquinone, 1-amino-2-methylanthraquinone,
2-methyl-1-nitroanthraquinone, 1-amino-2,4-dibromoanthraquinone (ADBAQ), and 1,3,8-
trihydroxy-6-methylanthraquinone (Emodin) (Doi et al., 2005). The results of these studies have
varied from completely negative to strongly positive regarding carcinogetic activity, with tumors
of several tissue types occurring in rats and mice. In addition to Disperse Blue 1, anthraquinone
and ADBAQ produced urinary bladder tumors in rats, and there was a suggestion of a bladder effect with 2-methyl-1-nitroanthraquinone. There was no effect on the urinary bladder in mice with any of these compounds, and essentially no effect on the kidney, despite the doses being comparable between the two species. Other tissues that were involved by one or more of these anthraquinones included liver, skin, and intestines in the rat, and liver, skin, forestomach, and lung in the mouse. The results with these studies have been complicated by the subsequent detection of significant levels of impurities, especially nitro-containing impurities that are strongly positive in genotoxicity assays. This has resulted in some controversy in the overall assessment of some of these compounds. What is clear, however, is that anthraquinones vary considerably in their carcinogenic potential, ranging from completely negative to strongly positive. However, it remains unclear as to whether any of the carcinogenic effects are due to genotoxicity, either partially or entirely, since there are toxicity issues in the induction of tumors of most of the tissue types in the rats and mice. However, especially with respect to the nitro anthraquinone compounds, there sometimes does appear to be a significant contribution by genotoxicity.

Of interest, is the finding that the anthraquinones which produce bladder tumors, such as Disperse Blue 1, were always associated with the production of calculi, although the compound with the strongest effect on the urinary bladder in rats was Disperse Blue 1. Only Disperse Blue 1 induced smooth muscle tumors.

VIII. OVERALL ASSESSMENT OF POTENTIAL CARCINOGENICITY IN HUMANS

Disperse Blue 1 has been tested thoroughly for carcinogenicity in an NTP two-year bioassay (NTP, 1986), and the findings in the rat were confirmed in a more focused investigation by Burnett and Squire (1986). The findings in both of these studies were the development of tumors of the urinary bladder at high doses, and that these tumors appeared to be associated with the presence of urinary calculi. Based on the criteria that are used for evaluation, the NTP (1986) has concluded that there is clear evidence of carcinogenicity in male and female rats for Disperse Blue 1. The International Agency for Research on Cancer (IARC) has evaluated Disperse Blue 1 with an indication that it is also a possible human carcinogen, again focusing on the urinary bladder as the target tissue (IARC, 1990). The NTP concluded that “Disperse Blue 1 is reasonably anticipated to be a human carcinogen based on evidence of malignant tumor formation in experimental animals,” in its Report on Carcinogens (Eleventh Edition, 2005). However, other reviewers have pointed to the close relationship between urinary calculi and the development of tumors, suggesting that a more realistic assessment of possible carcinogenic risk to humans is that this is a threshold response related to the formation of calculi, and that under the expected levels of use in hair dyes, Disperse Blue 1 does not pose a cancer risk for humans (Haws et al., 1994; Cosmetic Ingredient Review, 1995).

Overall, my inclination is that the data suggest that the tumorigenic effects of Disperse Blue 1 in rats are secondary to the induction of calculi, and therefore, that it is a high dose phenomenon only and is probably not relevant to humans at expected exposure levels, particularly given the low level of absorption through human skin. Bladder tumors did not occur in the mouse following Disperse Blue 1 administration, but there was an indication in mice of calculi and its
consequences. Mice generally are less responsive to the tumorigenic effects of calculi than rats
(Rodent Bladder Carcinogenesis Working Group, 1995; IARC Working Group, 1999). The dog showed no evidence of urinary tract effects, either neoplastic or non-neoplastic (Cosmetic Ingredient Review, 1995). However, I believe that there are several pieces of information not available to be able to conclusively conclude that all of the rat bladder tumors were induced only by the presence of urinary calculi. Based on these gaps in our knowledge, a more conservative approach I believe should be to assume a potential risk for carcinogenicity in humans, possibly independent of urinary tract calculi. Let me explain the basis for this hesitancy to absolutely conclude that it is safe.

Undoubtedly, there is a close relationship between the development of the urinary bladder tumors in the rat and the development of urinary tract calculi. However, there are aspects of the relationship that are of concern and not explained by the findings available in the literature. The two major issues concern the potential for genotoxicity of Disperse Blue 1 and the fact that in the NTP study a large portion of the tumors were of smooth muscle origin rather than epithelial.

The relationship of calculi to epithelial tumors of the urothelium, extending from the kidney pelvis to the urinary bladder, has been well documented in the literature (Rodent Bladder Carcinogenesis Working Group, 1995; Clayson et al., 1995; Cohen et al., 2002; Meek et al., 2003; Cohen, 2008). Difficulties that may be associated with a lack of a 100% correlation between calculi and tumors has been discussed, as well as the difficulties in extrapolating to humans. This has been detailed in a Rodent Bladder Carcinogenesis Working Group organized under the auspices of the International Life Sciences Institute (ILSI; Rodent Bladder Carcinogenesis Working Group, 1995) as well as a similar group of experts organized by IARC (1999). Both groups concluded that urinary precipitate and crystalluria do not pose a carcinogenic risk for humans, and urinary tract calculi may or may not pose a risk, and if so, it is related to a threshold response and only a small risk (Dominick et al., 2006). The major difference between rodents and humans with respect to carcinogenic risk evaluations based on urinary calculi is the length of time that these can be present in the organism (Rodent Bladder Carcinogenesis Working Group, 1995). The rat and mouse are horizontal quadrupeds, whereas the human is an upright biped. Because of the horizontal placement of the bladder in rodents, calculi can accumulate in the dome of the bladder and be present for the lifetime of the animal without producing complete obstruction. This was evident in the description of the animals in the NTP (1986) study and the Burnett and Squire (1986) study, where partial obstruction was observed but complete obstruction usually did not occur. In contrast, humans rarely have calculi present in the urinary tract for any length of time. This is because they usually lead to complete obstruction which produces excruciating pain in the patient and necessitates a trip to the physician for immediate removal, by surgery if necessary. There are several sites along the urinary tract for calculi to become obstructed in humans, beginning at the kidney pelvis-ureteral junction, the site where the ureter passes over the bony rim of the pelvis, the passage of the ureter through the bladder wall, and at the urethral outlet of the bladder. There are a few unusual circumstances where calculi can be present for prolonged periods of time in humans, such as bladder diverticuli or neurogenic bladder. Under these circumstances, there are a few, but not all epidemiologic investigations that show a slight increase in risk of bladder cancer in such patients. However, these patients nearly always have bacterial cystitis, a known risk factor for
bladder cancer in humans by itself. Thus, there is only equivocal evidence that calculi pose a cancer risk in humans.

Although some of the tumors produced in the NTP (1986) study and all of the tumors in the Burnett and Squire (1986) study were epithelial, either transitional cell or squamous cell, most of the tumors in the NTP study were of smooth muscle origin, either benign (leiomyoma) or malignant (leiomyosarcomas). The difficulty in concluding that Disperse Blue 1 is not carcinogenic to the rat urinary bladder based only on the association with urinary tract calculi is the fact that there are a large number of substances that have produced bladder cancer in rats and/or mice due to calculi, but this is the only example where tumors of smooth muscle origin (or mesenchymal tumors of any kind) have occurred (Oyasu, 1995; Clayson et al., 1995; Cohen et al., 2002).

Burnett and Squire (1986) suggest that the smooth muscle tumors arise as a consequence of a foreign body carcinogenesis process, similar to what has been described for subcutaneous sarcomas with thin films (Brand, 1975). As evidence, they point to the presence of crystalline and calculus material in the muscle wall in the bladder of some of the rats treated with Disperse Blue 1. In some instances, these solids were surrounded by a significant histiocytic infiltrate. This is similar to what is seen in foreign body carcinogenesis, but there are several issues that are not explained by this relationship. To begin with, in the subcutaneous tissue the tumors that are produced are primarily fibrosarcomas, and some may be related to the entity referred to as malignant fibrous histiocytoma. Smooth muscle tumors do not usually occur in this setting.

Another difficulty with the explanation by Burnett and Squire (1986) is the fact that the smooth muscle tumors occur in both the male and the female. In the male, most of the calculi appeared to be composed of the administered substance, Disperse Blue 1, or metabolites, and it may be that this provides a unique stimulus to the mesenchymal cells to induce smooth muscle tumors, in contrast to calculi that are composed of other substances in response to administration of a wide variety of chemicals. However, in their studies, they clearly document that in the female the major component of the calculi is calcium phosphate. Calcium phosphate precipitate, crystals, and calculi have been produced by a wide variety of substances when administered in high doses to rats, and in no circumstances have there ever been reported an increase in smooth muscle tumors (Oyasu, 1995). This includes circumstances where crystalline and calculus material have been reported in the wall of the bladder similar to what was seen with Disperse Blue 1. Even though the calculi were composed of calcium phosphate in the female rat, in contrast to calculi being composed of Disperse Blue 1 in the male rat, they both developed similar incidences of smooth muscle tumors and similar incidences of epithelial cell tumors.

An additional concern to the interpretation of the relationship of Disperse Blue 1-induced bladder tumors and calculi is the potential for genotoxicity. Disperse Blue 1 has been evaluated in a number of genotoxicity assays, and has shown positive results in many of these, albeit relatively weak. Whether or not this anthraquinone is truly genotoxic in vivo I believe has not been definitively determined. Although some of the genotoxicity positive results can be attributed to the contamination by the nitroanthraquinone metabolite, the definitive genotoxicity assay has yet to be performed using a highly purified TAQ, specifically free of the nitroanthraquinone. Furthermore, many of these assays were performed in the 1970s and 1980s,
and they need to be performed utilizing contemporary standards and interpretations. Evaluation of DNA adduct formation in the urothelium would be particularly useful to evaluate the DNA reactivity of Disperse Blue 1 in vivo. It is this uncertainty between the induction of smooth muscle tumors and the lingering possibility of genotoxicity that causes me to have the concern that I do. Until these can be addressed, the conservative approach is to assume that there is the potential for carcinogenic risk, albeit low.

Adding to this level of concern is the fact that other anthraquinones produced not only bladder tumors, but have also produced tumors in other organs in both rats and mice (Doi et al., 2005; IARC, 1982a; 1982b; 1990). The urinary bladder tumors have always been associated with calculi, but Disperse Blue 1 is the only one that is associated with smooth muscle tumors rather than epithelial tumors. Also, anthraquinones as a class produce tumors of a variety of different tissues in both males and females in both rats and mice, and the mode of action has not been clearly discerned for all of these. Potential genotoxicity is present for many of these, but not all, so this yet needs to be addressed.

It is clear that there are anthraquinones that are negative in both genotoxicity assays and carcinogenicity assays, so that anthraquinones as a class are not always genotoxic or carcinogenic risks for humans. Undoubtedly, some of the tumor findings in the rodents are arising secondary to non-genotoxic, proliferative mechanisms, and some of these may not be relevant to humans or may be only relevant at extremely high doses. Disperse Blue 1-induced bladder tumors likely fall into the latter category, but it is my concern, based on the above rationale, that there are sufficient gaps in our knowledge that one cannot conclusively state that it does not pose any cancer risk for humans.

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to be around during the team discussions so that will be the next step.

Now we are really going to shift gears from cocamidopropyl dimethylamine to talking about Disperse Blue. Dr. Cohen?

DR. COHEN: I'm going to talk today about the issue of carcinogenicity of Disperse Blue 1. You've discussed this in the past and there really is only a minimum of new data, but I'd love to give a different perspective on the data of the class of compounds of anthraquinones in general. I'm going to talk today first about the formulation and I think this is important because it's really not one chemical that comprises Disperse Blue 1. The main assay on which most of the discussion centers is the NTP bioassay which found that it caused bladder tumors. This was reinvestigated by Burnett and Squire in a paper that came out about the same time as the NTP report which looks at some specific aspects of the relationship of calculi to the tumors, also to look at the issue of
genotoxicity and also, too, put this into the perspective of other anthraquinones. Anthraquinone are actually quite widely used not only in cosmetics, but in a variety of industries. Then I'll give you my overall assessment, and I'd like to emphasize that this is my interpretation of the data.

To begin with, let me talk about what Disperse Blue 1 is. The main chemical is 1,4,5,8-tetraaminoanthraquinone. This comprises about 50 percent of the dye material itself, 20 percent of the composition is water and 30 percent is related chemicals, other anthraquinones. Importantly, one of the other anthraquinones is this triaminonitroanthraquinone that I've listed on the bottom. And this is important because the nitroanthraquinones really seem to have properties that are not as common to the other anthraquinones with other substituents. Then once you have all this composition of Disperse Blue 1 and you mix it 1-to-1 with ligonsulfonate dispersant, which is then what is used commercially so that the
This then is the NTP bioassay. As you can see here it's a high-dose phenomenon, 2,500 and 5,000 parts per million in male and female rats. What happened here is you get urinary bladder tumors. This is the only target site that was seen. It was associated with calculi and with an inflammatory response. The unique finding in this study that really is the unknown quantity are the tumors that I've highlighted in red here and that includes the smooth tumors that are leiomyomas and leiomyosarcomas. Unlike the transitional papillomas and carcinomas and squamous cell papillomas and carcinomas, we tend to lump the smooth-muscle tumors together as one category because they are a continuum. And if you put five pathologists together in a room you'll never get all five to agree which ones are benign and which ones are malignant, but they're all in this category. You'll see that most of them did
have the characteristics of malignancy which is an extension beyond the bladder.

The difficulty with this study was that it was associated only at the high doses, but the smooth-muscle tumors were the unique finding. In mice with the NTP study there were calculi, there were inflammatory reactions, but there were not tumors. One of the things to keep in mind is the relationship of calculi to tumor induction. The rat is probably the most sensitive species there is, the mouse is much less sensitive to the tumorigenic findings, and large animals like dogs, monkeys and humans it's still debatable whether calculi produce tumors at all.

Burnett and Squire then ran some additional studies looking at somewhat different protocols. The NTP study was with the substance without the dispersant. The studies with Burnett and Squire were primarily with the dispersant. They examined three different doses. The two lower doses went for 19 months because they didn't see anything, and at 1 percent for 6 months which
had to be stopped because of the large number of epithelial tumors, and, most importantly, they saw some partial obstruction and renal disease because of the urinary calculi. They also did some examination of these tumors and it's important to note that they did get tumors, they did get calculi, but the tumors that they saw were only epithelial tumors. They did not see any of the smooth-muscle tumors at all and I think this is an important distinction between the two studies.

They also did some short-term studies and some reversibility studies to try to narrow down what was going on. I think it's important that there has been some argument in the literature as to whether the fact that there's no 100 percent correlation between calculi being present at the terminal sacrifice and the presence of tumors. I think this really is a nonissue because calculi come and go in the urine primarily because they can shrink in size and be voided and then form again, and some can actually be lost during the processing because they'll dissolve in
the solution. I think just the fact that a large proportion of these animals had calculi that one can really assume that during the lifetime of those animals they all had calculi at the highest dose and I don't think that's an issue that needs to be examined further. It's been looked at with a number of chemicals, including things like uracil and phosphorylated-1, which is a pesticide, where at the terminal sacrifice only a proportion of the animals would have calculi. But if you look earlier on in the short-term study, a 100 percent of the animals will have calculi, so I don't think that's an issue.

In short-term examinations Burnett and Squire looked at both gavage administration at a very high dose as dietary administration. Somewhat interestingly, when it was given by gavage at a gram per kilogram for three days, there was accumulation of the substance in the kidney and this is a kind of chemist's dream because you don't even need an instrument to analyze. You look and if it's blue it's got the
dye in it, if it's not blue it doesn't have the dye in it. It's a pretty easy analysis. But the key is that there was urothelial hyperplasia in the kidney pelvis. And for those who are not familiar with lower urinary tract anatomy, the kidney itself is a tubular structure. And then the collecting part of the kidney, which is the kidney pelvis, is lined by a lining that's called the urothelium, and that's the same lining that then continues down the ureters and the urinary bladder so that essentially from the kidney pelvis to the urethra is all one organ system called the urothelium.

They noticed that there was hyperplasia in the kidney pelvis and some evidence of inflammation, but there were not any changes in the bladder in the short term with the gavage. However, dietary administration for four days led to the formation of urinary solids, again urothelial hyperplasia, but this time not only in the kidney pelvis in the bladder and they were able to show that they got exactly the same
results in the diet whether the dispersant was or wasn't used. They concluded, and I think it's true, that it's the Disperse Blue 1 itself that's causing the changes and not the dispersant and the dispersant probably don't have an effect here.

Because of the large number of animals that were dying by six months of the experiment, they stopped treatment at this time and took a large number of the animals that had already formed calculi and surgically removed them and kept those going as well as some animals that they didn't remove the calculi to see if there were any differences and to see if they would regress. It turned out that in the males when they took the calculi out, the bladders returned essentially to normal except for a few animals that already had tumors and they subsequently still had tumors when they died. The females interestingly, though, increased the number of calculi that were present. And this was probably related to the fact that the calculi that formed in the females in these studies when they were on diet and after they were
on diet actually were composed of calcium phosphate rather than the dye itself, whereas in the males it was composed of the dye. So there is a distinction here between males and females and yet the response tumorigenic-wise, hyperplasia, inflammation, and everything else was really identical between the two which would suggest that it's really the calculi that's doing the damage and not the chemical itself. However, there are some difficulties there.

Interestingly, though, the tumor incidence at the end of this experiment, whether they had done surgery to remove the calculi or not, the tumor incidence was the same in both the males and females in the surgically removed versus the non-surgically removed. Again, the number of animals involved here is pretty small, so they're going to be able to detect relatively minor differences, but I thought that was a bit of an unusual change. In the females at least, the continued presence of the calculi is probably related to the fact that when they removed these
stones they leave sutures behind and sutures are well-known nidus for the continued formation of calculi.

Burnett and Squire again noticed that the calculi in the males were composed of the blue dye, the Disperse Blue 1, where in the females it was predominantly calcium phosphate, which is a common constituent of calculi in the rat in contrast to humans where most of the calcium stones are calcium oxalate. The tumors that were associated with the calculi in the Burnett Squire study, however, were only epithelial. They didn't see any smooth-muscle tumors. They conjectured that the reason for their findings compared to the NTP study was, one, that they included the dispersant; that it was different doses; and three, they didn't have as long an experiment. The NTP study is a 2-year study and the Burnett and Squire study is a 19-month study.

They also pointed out, and this has been pointed out in the NTP study as well, that there were calculi present not only in the lumina of the
bladder, but actually penetrating the wall of the bladder. And in some of these animals there was not only an inflammatory reaction, but a histiocytic response. So their conjecture that even though they didn't see any smooth-muscle tumors was that the smooth-muscle tumors that occurred with the NTP were essentially a formed body reaction type of sarcoma that has been seen for 30 or 40 years in mouse and rat subcutaneous administration. The difficulty you have there is that when you put them at least in the subcutaneous tissue, the tumors you get are primarily fiber sarcomas or malignant fibrocystic sarcomas, they're never of smooth-muscle origin.

The biggest difficulty with the study is that there have been dozens and dozens and dozens of different chemicals of different types producing calculi in rat and mouse bladders and they've never been associated except in this one instance with smooth-muscle tumors. They're always epithelial; in fact, most often squamous. Also there's a good mix of the traditional cell,
but this is the only example that I'm aware of in the literature and not in the literature that has produced smooth-muscle tumors, especially in such a high incidence. Smooth-muscle tumors of rat bladders are rare, and they're also rare in humans, by the way.

What about the genotoxicity of Disperse Blue 1? Here's where some of the complications come in. It's been positive and it's been negative. It's been positive with the S-9 and without the S-9. It's been positive Ames assay, the mouse lymphoma assay, the chrome ab study and the sister chromatid exchange test. These are all in vitro. And some of the difficulties that you have include, again, this is Disperse Blue 1, this is not a pure chemical, and 6 percent of the chemical that's being analyzed is the nitroanthraquinone, nitro aromatics in general, and extremely potent mutagens in in vitro systems. They're not as uniformly positive in in vivo systems. The difficulty we have with Disperse Blue 1 is we really don't have a decent evaluation
of the in vivo genotoxicity. The only study I'm aware of in vivo of genotoxicity was a hamster chrome ab study, which has, I think, some difficulties in interpretation, but it was at least negative. Again, there are not a lot of hamster chrome ab study to compare this as well and there are some difficulties with it furthermore.

Again, these were all in vitro assays. The results are weak to equivocal. They are positive with and without the metabolic activation, which I attribute to the fact that there's nitroanthraquinone present. Nitroanthraquinones don't need metabolic activation because the bacteria themselves and mammalian cells can reduce the nitro group quite readily and you end up with quite reactive compounds.

The other is that for many of these studies, and I didn't have them available in detail to look at, but they were all performed in the 1970s and 1980s at a time when there wasn't as
clear-cut standards for their performance, and, more importantly, there weren't as clear-cut standards for their interpretation. So that there is that difficulty in interpreting these as well.

Obviously what you're interested in is the dermal application of the compound. There have been some studies in vitro with rat and human skin systems looking at dermal absorption and, as expected, there is minimal dermal absorption across the epidermis. There has been a topical carcinogenicity in mice. This was negative. I think this is not particularly helpful in your deliberations primarily because the exposure level would be relatively low, the absorption is low, and somewhat peculiarly they didn't examine the bladders in any of the animals which is essentially the target site. You also have a two-year oral bioassay, though, at very high doses which was negative despite the presence of calculi.

Where does this fit then with other anthraquinones? The NTP has undertaken the
examination of seven different anthraquinones in their two-year bioassay protocols both in rats and mice. And these are the seven that are listed in the Doi, et al., paper if you want the details that list organ by organ. What I think is of interest is that they looked at a variety of substituents on the ring, including the anthraquinone itself. As expected, many of these were positive in gene tox studies and some were negative in gene tox studies.

It's important to note that, at least in the literature from what I could find, there have been approximately 90 anthraquinones examined in gene tox studies of one sort or another and approximately a third of them are positive. The one group of the anthraquinones that you uniformly get positive results on is if there is a nitro substituent anywhere in the rings. In contrast, amino substituents, hydroxyl substituents, methyl substituents, bromo substituents, chloro substituents, have variable responses in genotoxicity. Similarly, in the carcinogenicity
results, there is quite a range of results as well.

With genotoxicity, again, you get positive results and you get negative results. None of these have looked at in detail with respect to contaminants and how much of a role they play, and in some of these I'm almost sure that it's a contaminant that's causing the positive response and not the compound itself, particularly since the contaminants in some of these substances is at appreciable levels.

As for carcinogenicity, it's interesting that out of the seven that have been looked at in the NTP bioassay, three of them produced tumors. Out of the three that produced bladder tumors, they were always associated with calculi, but, again, of interest is the fact that only Disperse Blue 1 produced smooth-muscle tumors and none of the others produced any smooth-muscle tumors. They were the more traditional findings that they produced, squamous cell and urothelial type tumors including anthraquinone itself. With
anthraquinone you need to keep in mind that there is a substantial contamination of different nitroanthraquinones that were present in the material that was analyzed.

Of interest, though, is that out of all of these at least one of these compounds was positive in male or female rats or male or females mice and frequently in multiple ones. Of importance, I think, in extrapolating the findings of this is that not only was the bladder a target for some of these and only in the rat, but other tissues were targets as well, including the usual suspects such as liver and lung, but also interestingly for some of these was colon cancer, so that there is quite a variety of different tissues involved.

Going back to Disperse Blue 1 and the bladder tumors, again they're always associated with calculi, it's a high-dose phenomenon only and there is the issue of questionable genotoxicity, which I would attribute to the nitroquinone, but until somebody actually looks at the pure
substance or analyzes this further, I think one can't definitively state that at this point. Again, the tumors were associated with the calculi. But as I indicated earlier, this is the only chemical I'm aware of that produced smooth-muscle tumors.

I find this interesting because the lesions in the females, the calculi in the females, were calcium phosphate stones. And there are a whole bunch of different chemicals that produce calcium phosphate stones in rat bladders and they don't produce smooth-muscle tumors, so that there is still something about the fact that these animals, when they penetrate the bladder with these stones -- and keep in mind that if you get a stone into the wall of the bladder it's had to go through the urothelium and you've got an ulcer. And it's kind of like just cutting yourself on the skin, which is the same process in the bladder. To get there it means that the underlying smooth muscle and connective tissue is being exposed to the dye itself whether it's in
the form of the calculus or the soluble dye is present.

Then the other part is that with the anthraquinones at least the bladder tumors are always associated with the calculi. Again, as to genotoxicity we don't have definitive evidence. There is the potential, and this is strictly conjecture because there is no evidence that it actually happens, that you get N-hydroxylation of the amines. Personally, I would be very surprised with the 1 and 4 substituents that you have on these that you would get N-hydroxylation. It's like the comparison of 1 naphthylamine versus 2 naphthylamines where the 1 doesn't N-hydroxylate and the 2 does and these are essentially the 1 amines. I would doubt that, but, again, with the in vivo we don't really have definitive information on the in vivo toxicity of this compound or, for that matter, on virtually any of the anthraquinones. There are very many of them. There has been no assessment of DNA adduct formation which, to me, would give us the ultimate
conclusion as to whether this really is a meaningful geno toxin or not.

Then there is the issue of nitroanthraquinone contamination. And certainly 6 percent contamination of the Disperse Blue 1 with this much nitroanthraquinone is more than enough to explain the genotoxicity in the compound in the in vitro systems.

As to other anthraquinones, again, there are various ones that are positive in rats and/or mice, and other organs have been involved besides the bladder so that the bladder is not the only tissue involved with these chemicals. It just happens to be the only one with the Disperse Blue 1.

My overall conclusion is that, yes, Disperse Blue induces bladder tumors. The issue that we're still left is what do these smooth-muscle tumors in the wall of the rat mean in the NTP study? We really don't have a mode of action for these tumors. This is the only example we have.
As to genotoxicity we don't have any real data for this in vivo or actual adduct formation. Then we have the issue of the nitroantrahraquinone contaminant.

I'd be happy to answer any questions.

DR. SLAGA: You stated that Burnett and Squire didn't get any smooth-muscle tumors in their experiment, though. Right?

DR. COHEN: Correct. They really couldn't come up with an answer as to why. They thought most likely it's the time of the experiment, but the reality is that smooth-muscle tumors were seen in shorter times than 19 months in the NTP study. I've reviewed some of the slides from the NTP and there is question that they are smooth-muscle tumors and many of the animals had both smooth-muscle and epithelial tumors. There is still a peculiarity there.

DR. BELSITO: In your bullet there you say human relevance can't be fully assessed, but to step back to your talk you said that dermal penetration of this is quite low. So looking at
the doses orally even under a worst-case scenario that caused the calculi, you really wouldn't expect to achieve those types of levels from a hair dye use.

DR. COHEN: I agree. I don't think you would achieve those significant levels. I think the real issue that we come down to is that if the tumors truly are related only to the calculi, then this is really a nonissue for human exposure and human risk and that's probably true. The problem is I just don't think you really have all the data you need to come to that conclusion mainly because of the issue that you still have this nagging suspicion that there is genotoxicity which would complicate things a bit. And the other is that calculi don't produce smooth-muscle tumors. This is the only example that's been known of any type of chemical.

DR. MARKS: Let me clarify. We have one study that shows smooth-muscle tumors in rats and that's it? It's never been reproduced other than that one NTP study in 1986? Is that correct?
DR. COHEN: Correct.

DR. MARKS: And other than Burnett and Squire also in 1986, has anybody tried to reproduce that since 1986?

DR. COHEN: Not that I'm aware of.

DR. MARKS: This is of course all animal data. Do we have any epidemiologic data to suggest there is an increased incidence of smooth-muscle tumors in humans?

DR. COHEN: Not that I'm aware of, and since it's such a rare type of tumor, I think if centers saw a couple of cases in a year of smooth-muscle tumors alarms would go off. So as far as I know there has not been an actual epidemiologic study of this, I would expect that there hasn't been an increase. These are very uncommon tumors.

DR. ANDERSEN: In terms of the epidemiology data, certainly bladder tumors have been looked at. The question is going to be do those studies have any subcontext to them enough to resolve the question of tumor type?
MS. SKARE: We'd probably need to go back and look at that more carefully, but what I would say is that in most of the epi studies you get a categorization of urinary bladder tumor from the pathologist and the predominant form would be transitional cell carcinoma, but probably any type of bladder tumor would have been included in those epi studies.

DR. BELSITO: I guess in terms of epidemiology studies for anthraquinones, probably the relevant industries wouldn't have been the hair-dressing industries that we've looked for hair dyes, but more the textile or the printing industries. Is there any known about bladder cancers in those industries?

DR. COHEN: There have been some epidemiology reports in both of those industries of increased risk but it's never been known what chemicals specifically, and it's not been uniformly found and sometimes there's a slight increased risk and sometimes there's increased risk with it. But both of those industries,
particularly the textile industry, has been. These were done several years ago where it was thought because of exposure in the 1940s and 1950s that a number of aromatic amines where known to be included in the dyes used back then, especially benzidine dyes, which obviously aren't used any longer.

DR. SNYDER: Sam, is it common for humans with urinary calculi to have migration of the calculi into the wall of the bladder?

DR. COHEN: It's not common, but it occurs.

DR. SLAGA: Could you summarize the differences between the NTP study and Burnett and Squire in terms of dosage and length?

DR. COHEN: The top dose in the Squire study was essentially 1 percent of the diet. In the NTP study, 2,500 and 5,000 PPMs were the positive doses. I've got it back here. Here are the doses in the NTP study that were there. And they also used the same strain of animals from the same source, the same diets and the same age at
the beginning. I think the major difference between the two studies would have been the time of administration. In the Squire study, he only administered it for 6 months at the high dose that gave tumors because and that's essentially 10,000 PPM, and then his next lowest dose was 1,000, which was negative, so that the 1,000 that he was able to take to 19 months was negative, but he was really only able to administer compound at the effective dose for 6 months and then kept the animals alive for up to 19 months, whereas in the NTP study they went on for a total of 24 months. I think that's your biggest factor. And mesenchymal tumors in general in organ systems like this will occur much later in the lifetime of the animals and that's been pretty much true as with hemangiosarcomas in various tissues. They're a very late-arising tumor in animal models.

DR. HILL: Having had occasion to both purchase aromatic amines and even synthesize them in the lab, I can assure you that the amounts of nitro impurity can vary around quite a bit so that
not knowing anything about whether the same lots were used and the nitro contaminant is, in fact, the major player, which I could easily envision. I'm wondering in terms of the commercially available Disperse Blue now how closely is that monitored? Does it vary around a lot?

DR. COHEN: From what I understand, Disperse Blue is no longer commercially available because it was withdrawn in Europe or banned in Europe. And someone from the industry will have to give you more details, but as far as I know it's not commercially used anymore because of the remaining questions.

DR. ANDERSEN: Thank you very much. I hope you're going to be around when the teams get into discussing this so we can take the next step.

Now we're going to have our last speaker, which will involve first an equipment change so that we'll take a short timeout while we get this set up.

(Recess)

DR. BERGFELD: If we could reassemble,
Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: John Bailey, Ph.D.  
Industry Liaison to the CIR Expert Panel

DATE: August 23, 2010


p.3 - At what concentration was Disperse Blue 1 a moderate sensitizer in guinea pigs?
p.3 - What type of tumors resulted in the equivocal results in male mice?
p.4 - In the summary of the Risk Assessment section, it would be helpful to indicate the type of exposure, e.g., hair dye use, for which the estimated lifetime daily dose of Disperse Blue 1 was estimated.